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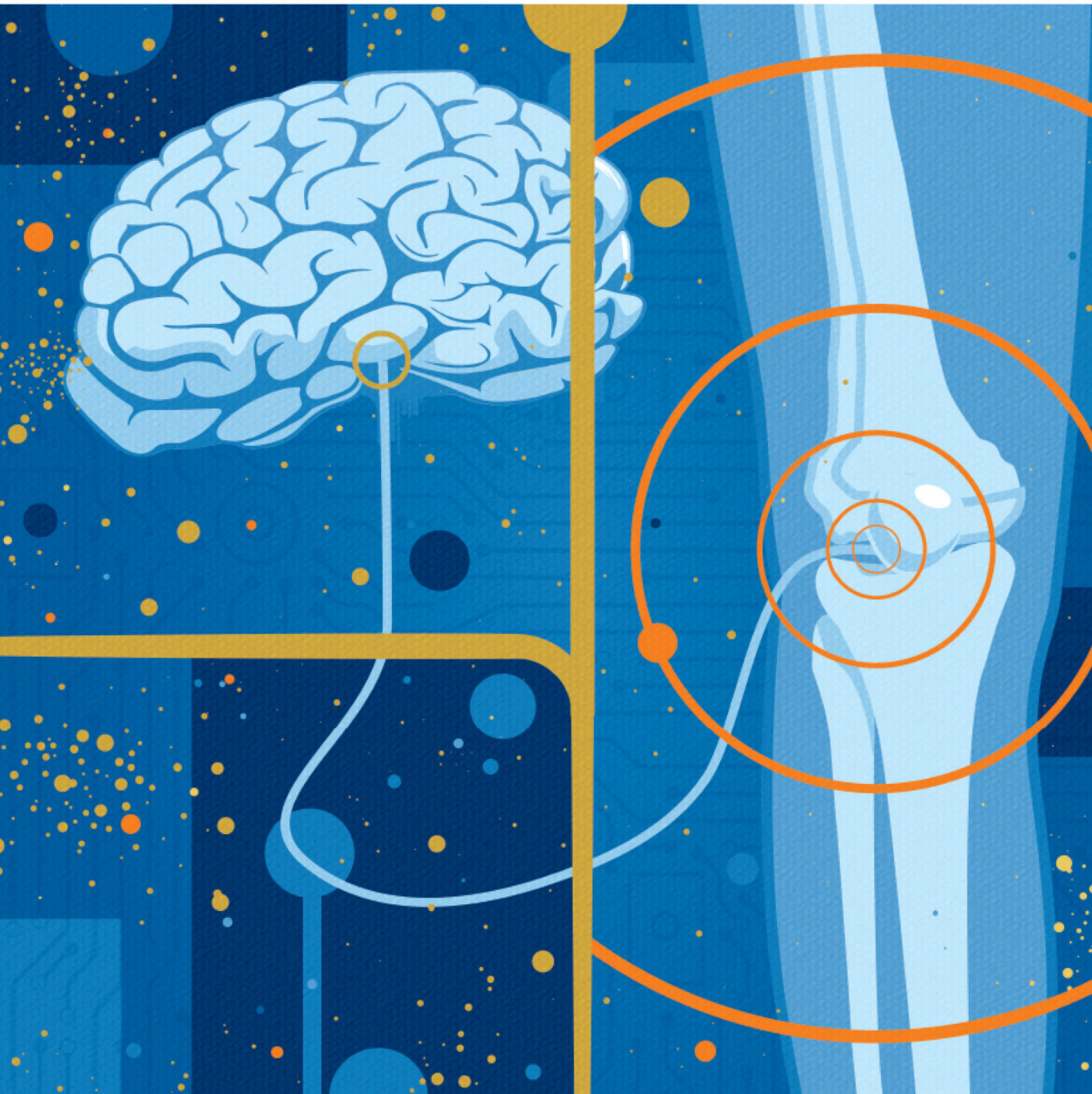

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Sex- and gender-based personalized medicine in rheumatology

Elizabeth R. Volkmann & Carol Feghali-Bostwick

Although autoimmune rheumatic diseases are more prevalent in women than men, few clinical trials report findings on the basis of sex and gender. Future clinical trials should report sex and gender differences in treatment and safety outcomes in a standardized manner to improve outcomes for all patients.

Innumerable aspects of human health are influenced by sex, including disease prevalence, prognosis and treatment responsiveness. Sex differences are particularly pervasive in autoimmune rheumatic diseases owing to their inherent predilection for affecting women more than men. The journey of women living with an autoimmune rheumatic disease can differ substantially from that of men, beginning with obstacles encountered in obtaining the correct diagnosis because of unconscious bias. Women might also experience varying susceptibility to medication toxicity compared with men, and adverse events are more common and more severe in women than in men, which could in turn affect adherence and quality of life.

Improved awareness and understanding of how sex differences and gender biases affect people living with autoimmune rheumatic diseases has the potential to improve outcomes for all patients; however, the implementation of sex-specific personalized medicine in autoimmune rheumatic diseases is only possible if clinical and translational research studies commit to studying these differences. Surprisingly, prior to 1994, women were excluded from all clinical trials in the USA, making studying sex and gender differences in efficacy and safety outcomes of therapies approved for autoimmune rheumatic diseases prior to this date a challenge. Although women have been included in interventional clinical trials for the past 30 years, few trials have prioritized the reporting of sex and gender differences in the publication of results that support the success of new drug applications in rheumatology.

Although regulatory agencies require clinical trials to include women, there is no consensus on how to regulate the reporting of sex-specific comparisons in rheumatology clinical trials. Data from post-hoc analyses have illuminated important sex differences in efficacy endpoints. For example, a systematic review and meta-analysis of 54 randomized controlled trials (RCTs) (n = 11,514 women and n = 11,107 men) in psoriatic arthritis demonstrated that 18 RCTs reported efficacy endpoints by sex and only 2 reported safety outcomes by sex[1]. Sex differences in treatment response varied according to the drug class analysed, pointing to a biological basis for the observed sex differences[1].

In rheumatoid arthritis (RA), studies report higher remission rates in men than women across different drug classes[2]. Similarly, in axial spondyloarthritis, women are less likely to achieve efficacy outcomes on advanced therapies than men[3]. By contrast, in systemic sclerosis, a post-hoc analysis of two RCTs for interstitial lung disease (ILD) revealed that women had improved outcomes compared with men when treated with either cyclophosphamide or mycophenolate mofetil[4]. In some rheumatic diseases, such as systemic lupus erythematosus, the study of sex and gender differences might be more challenging as women comprise the overwhelming majority of patients in RCTs.

Data on sex and gender differences in safety outcomes from RCTs in rheumatic autoimmune diseases are even more sparse than efficacy endpoints. In a post-hoc analysis of pooled data from four RCTs evaluating the efficacy and safety of nintedanib for ILD, two of which included patients with connective tissue disease-associated ILD, women were more likely to experience nausea, vomiting, liver enzyme elevations, dose reductions and treatment interruptions than men treated with nintedanib[5]. In a post-marketing safety surveillance study of tofacitinib over 9 years in patients with psoriatic arthritis and RA, adverse event reports were more commonly submitted for women than men[6]. The disease prevalence of RA is 2 to 5 times higher in women than men and might explain some of the differences observed in adverse event reporting in this study; however, the disease prevalence of psoriatic arthritis is similar in men and women, suggesting that more adverse events occur in women than men with this disease. Standardizing the reporting of biological sex and self-reported gender differences in post-marketing safety surveillance studies represents a promising avenue for understanding how specific rheumatic disease therapies affect men and women using real-world safety data.

Biological variables might underlie some of these differences. Sexual dimorphism has been noted in drug absorption, bioavailability, distribution, metabolism and elimination. Differences in how men and women respond to treatments could result from physiological differences such as weight, height and blood volume, as well as differences in pharmacokinetics and pharmacodynamics[7,8]. In addition, sexual dimorphism leads to differences in adverse events, dose, dosing regimen and treatment intervals. For oral drugs, absorption might be different in men and women because of differences in gastric pH (which is more acidic in men than women), intestinal motility and gastric emptying (both of which are higher in men than women), or other physiological differences such as higher renal clearance in men than women. For dermally injected drugs, women might require different doses than men as they have higher subcutaneous lipid content.

Drug metabolism can also differ with sex owing to differences in drug metabolism enzymes and transporters in men and women; sex differences are reported in the expression of hepatic drug metabolizing enzymes. In addition, the gut microbiome is different between the sexes and can affect drug absorption. Sex hormones, such as oestrogen, can alter hepatic enzyme activity, and the use of oestrogen-based contraceptives, often a requirement for women enrolled in clinical trials, can confound outcomes.

Box 1 | Reporting of sex and gender differences

We provide a hypothetical example of how sex and gender differences in study participants could be reported, using rheumatoid arthritis (RA) as an example. The data used in this example are fictional and for illustrative purposes only.

Special considerations related to sex and gender: RA affects more women than men (ratio of 2–5:1).

Overall representativeness of this trial: The participants in the present trial demonstrated the expected ratio of women to men (ratio of 3:1).

How sex and gender were classified: Biological sex was reported by participants based on their response to the following question: "What was your sex assigned at birth?" Options included female, male or intersex. Gender was reported by participants based on their response to the following question: "What is your gender identity?" Options included woman, man, nonbinary or prefer not to say.

Sex and gender differences in baseline characteristics: Demographic characteristics were similar for women and men in this trial, with the exception of age (mean age of men was 45 years; mean age of women was 55 years). Prior or current tobacco use was more common in men than women (45% versus 25%, respectively). Baseline disease activity scores were similar between men and women based on the Clinical Disease Activity Index.

Sex and gender differences in primary outcome: The proportion of women and men who achieved remission based on the Clinical Disease Activity Index at 12 months was 20% and 40%, respectively.

Sex and gender differences in safety outcomes: Serious adverse events were rare in women (3%) and men (2%). Among adverse events, nausea occurred more commonly in women (30%) than men (15%).

Implications of the findings as it relates to sex and gender: In the present trial, a treatment effect was observed for all participants, with a higher remission rate noted in men than in women. The safety profile of the investigational agent was similar between men and women, with the exception of nausea, which occurred more commonly in women.

In addition to differences in drug metabolism, the mechanisms by which specific medications affect physiological responses can vary. For example, anti-inflammatory drugs are often used in rheumatic diseases; mechanisms by which NSAIDs work are different in men versus women. In fact, female sex is associated with NSAID-related adverse effects such as upper gastrointestinal symptoms and ulcers[9]. Aspirin metabolism is also different in men and women; slower clearance in women results in a longer half-life and greater bioavailability of acetylsalicylic acid[10].

Understanding sex and gender differences in the response to therapy in patients is crucial for designing safe and effective treatments and can inform clinical management of patients with rheumatic diseases. In Box 1, we provide a hypothetical example of a standard approach to reporting sex and gender differences in RCTs. This approach would require investigators to disclose known sex and/or gender differences in the prevalence of the disease under study and reflect on the representativeness of their cohort with respect to sex and gender. Details on how information on sex and gender was obtained from study participants should also be reported. The reporting should also address sex and/or gender differences in baseline characteristics of the cohort, as well as the primary efficacy outcome. Notable sex and/or gender differences in safety endpoints should also be provided. Journals should require that this information on sex and gender accompanies each trial manuscript in the same way that authors are required to disclose relevant financial conflicts.

Inclusion of these data will not only illuminate important sex and/or gender differences in study outcomes but also generate the data necessary to perform meta-analyses and systematic reviews of sex and/or gender differences across drug classes in specific diseases. For early phase studies, sponsors should be encouraged to report pharmacokinetic and pharmacodynamic data by sex.

In summary, consideration of sex and gender can improve patient outcomes and inform personalized and tailored therapeutic strategies. Although much of the understanding of sex differences in response to treatment has resulted from post-hoc analyses, prospective studies will provide more detailed insights into these differences. It is imperative for the rheumatology community to make the reporting of sex and gender differences compulsory in all trial publications. The data on sex and gender are readily available; it is the responsibility of investigators and the wider medical research community to ensure that these data are disseminated properly and without bias to improve outcomes for all individuals living with autoimmune rheumatic diseases.

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Competing interests

E.R.V. consults for Boehringer Ingelheim, GSK and AbbVie, all of which are outside the scope of this article.

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Lupus ABC spearheading a new era of collaboration to advance lupus drug development

Hoang Nguyen & Teodora P. Staeva

Despite having a robust drug development pipeline, lupus remains far behind other rheumatic and autoimmune conditions for which dozens of targeted therapies have been developed. Addressing the pervasive, long-standing challenges impeding the field requires a paradigm shift and a patient-powered, community-wide approach, exemplified by the Lupus Accelerating Breakthroughs Consortium (Lupus ABC).

Systemic lupus erythematosus (SLE) — a highly complex autoimmune disease with varied clinical manifestations[1] — is an area of major unmet medical need and high disease burden[2]. Treatment options are limited and often confined to decades-old drugs with substantial adverse effects[3], underscoring the urgent need for safer and more effective treatments. Despite extensive clinical research, only three therapies developed specifically for SLE or lupus nephritis have been approved in the past 70 years, and none for primary cutaneous lupus erythematosus (CLE). The extraordinarily high failure rate of lupus trials has led to a consensus in the field that the existing trial designs and outcome measures are inadequate to capture the efficacy of experimental drugs in the context of the vast heterogeneity of lupus[4]. Further exacerbating these issues, the perspectives of patients have not been adequately incorporated in the drug evaluation process. These complex challenges require a collaborative approach that engages all stakeholders including regulators, patients, drug developers and physician-scientists to develop shared solutions.

Public–private partnerships (PPPs) have been effectively used for decades to address bottlenecks in drug development beyond the capabilities and resources of any single entity[5]. Thus, they provided an ideal approach to unite the lupus community under a new PPP, the Lupus Accelerating Breakthroughs Consortium (Lupus ABC), with the core mission of uniting the lupus community to expedite the development of safer and more effective treatments for people with lupus.

Establishing a new collaborative approach in lupus

Launched in March 2023 as a collaboration between the Lupus Research Alliance (LRA, the largest global non-profit, non-governmental funder of lupus research) and the US Food and Drug Administration (FDA), the Lupus ABC has three main aims. First, to convene the lupus community and leverage the collective expertise of regulators, people with lupus and their advocates, academic researchers and clinicians, industry partners, professional societies, the National Institutes of Health (NIH) and patient advocacy organizations. Second, to develop timely initiatives to overcome regulatory hurdles in lupus drug development by using an open, collaborative, pre-competitive approach. Third, to ensure that the experiences, perspectives, needs and priorities of individuals living with lupus are captured and meaningfully incorporated into lupus drug development.

Instrumental to achieving this mission is a robust organizational structure that provides balanced representation of members and effective operation through two central governing bodies, the Research Committee and the Lupus Voices Council (LVC), which set the strategic and patient priorities of the Lupus ABC, respectively. Projects within the PPP are conducted by working groups empaneled by the Research Committee and the LVC (Supplementary Fig. 1).

Leveraging the power of the Lupus ABC to hasten progress

In its first two years, the Lupus ABC has made notable progress in engaging the lupus community and in operationalizing and implementing projects (Fig. 1). At its launch, the Lupus ABC attracted 32 member groups from academia, the FDA Center for Drug Evaluation and Research (CDER), the NIH, professional societies, patient advocacy organizations and 17 industry partners. Within a year, total membership had increased to 49 member groups, including the FDA Center for Biologics Evaluation and Research (CBER) and 33 industry partners[6]. Four projects were launched during that first year, each of which reached a key inflection point within 12 months supported through regular oversight by the Research Committee. These early advances demonstrate the ability of the Lupus ABC to convene and leverage diverse expertise, including the patient voice, to collectively address complex challenges and expedite advancements in lupus drug development.

In line with Lupus ABC's second goal, the Research Committee and the LVC selected projects primarily on the basis of pressing clinical and patient needs and requirement for regulatory input. Recognizing the major limitations of current outcome measures, which were not specifically developed to capture treatment benefit in lupus trials, the PPP embraced, from the start, the need for more effective and fit-for-purpose instruments and an emphasis on patient-reported outcomes (PROs).

The first project of the Lupus ABC was a partnership with the pre-existing Treatment Response Measure for SLE (TRM-SLE) initiative[7], the focus of which is the development of a novel, fit-for-purpose outcome measure for SLE trials. Recognizing the potential of this project, the Lupus ABC partnered with TRM-SLE to provide crucial regulatory

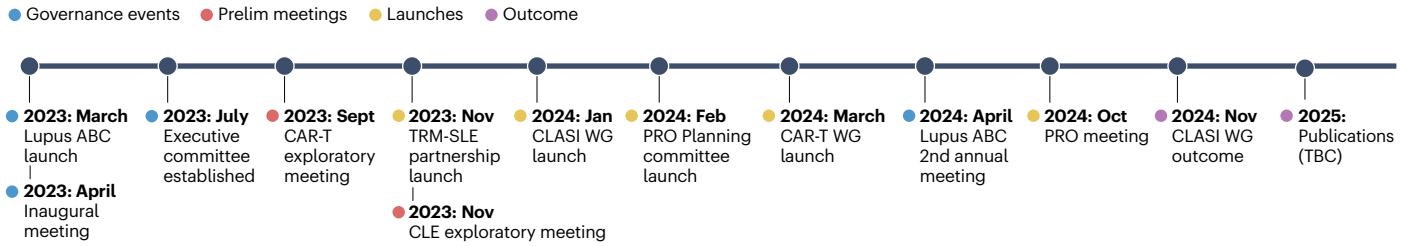

Fig. 1 | Timeline of Lupus ABC milestones during its first 2 years. Since its inception in 2023, Lupus ABC has partnered with the pre-existing Treatment Response Measure for Systemic Lupus Erythematosus (TRM-SLE) initiative and launched projects on Cutaneous Lupus Erythematosus (CLE) Area and Severity Index (CLASI), chimeric antigen receptor (CAR)-T cell therapy for lupus and patient-reported outcomes (PROs). WG, working group.

insights. The most notable achievement of the Lupus ABC so far is its influence on drug development for CLE, for which there is currently no approved therapy, owing in large part to the lack of a universally endorsed clinical outcome measure. In 2024, the Lupus ABC launched a working group involving all key CLE partners to evaluate the use of the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI)[8] as a primary instrument to establish drug efficacy in CLE clinical trials, given the large amount of published data and ongoing efforts to validate this outcome instrument[9]. Real-time feedback from CDER and the sharing of unpublished data from academic and industry partners, which were made possible by the PPP, enabled crucial consensus-building on the use of CLASI in this setting. The importance of this milestone, which was achieved in an impressively short time, cannot be understated as it provides a critical tool to evaluate treatment responses in CLE and creates a path for the approval of urgently needed therapies.

Recognizing the transformative potential of chimeric antigen receptor (CAR)-T cell therapy for lupus[10] and bolstered by the addition of 16 more cell therapy companies into the PPP, the Lupus ABC launched a CAR-T cell working group in 2024, enabled by Lupus ABC's expanded collaboration with the FDA to include CBER (Fig. 1). This project has now achieved its first objective of defining the main considerations for advancing the safe use of CAR-T cell therapy for SLE. The rapid launch of this working group and the expansion of the PPP to include CBER demonstrate Lupus ABC's agility in responding to emerging scientific opportunities and the eagerness of the community to adopt a collaborative approach. We anticipate that the outputs of the TRM-SLE, CLASI and CAR-T cell projects will be published in 2025, setting the stage for the next phase of the working groups.

Integrating the patient's perspective into the lupus drug development process is one of the founding principles and key priorities of the Lupus ABC. Establishing an impactful project on lupus PROs, however, requires a dialogue among the key experts and partners to assess the current state of the field and to identify the most timely opportunities to advance lupus PROs for regulatory decision-making. Once again, Lupus ABC's unique convening power enabled such a forum, resulting in the development of a robust pipeline of potential projects, which are currently being prioritized to empanel a new working group on lupus PROs later in 2025. Lupus ABC projects, especially the PROs, highlight the crucial contribution from patients, through the LVC, in shaping research priorities and the central role of the PPP in amplifying this perspective.

Drug development in lupus is hindered not only by outcome measures, but also by the current trial design framework, which is reliant on the use of restrictive entry criteria and the inclusion of heterogenous populations, as well as variable use of background medications and the lack of predictive and surrogate biomarkers[2]. These limitations, further compounded by the high rates of response to placebo or standard care, impede the accurate assessment of drug efficacy. Addressing these issues is a key priority for the Lupus ABC, and the PPP will be considering projects proposed by the broader community to further address these challenges.

The Lupus ABC illustrates the power of a PPP approach rooted in broad community engagement and the integration of diverse perspectives in real time. The Lupus ABC provides unique value to the lupus community and is poised to propel further lupus drug development. More broadly, the Lupus ABC offers a blueprint for the establishment of PPPs with the FDA in rheumatic, autoimmune and autoinflammatory diseases.

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Competing interests

The authors declare no competing interests.

Additional information

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COVID-19

A role for TGFβ and EBV in MIS-C pathogenesis

Multisystem inflammatory syndrome in children (MIS-C) is a rare complication of infection with SARS-CoV-2, usually occurring 4–8 weeks after infection. Previous studies have correlated severe COVID-19 in adults with increased levels of the immunosuppressive cytokine TGFβ, and MIS-C with the expansion of T cells, particularly a T cell subset that expresses the Vβ21.3 T cell receptor β chain variable region (TCRVβ21.3).

A study now published in *Nature* analysed peripheral mononuclear blood cell (PBMC) samples from children with and without MIS-C after SARS-CoV-2 infection and associated the increased levels of TGFβ in MIS-C with T cell dysfunction and reactivation of Epstein–Barr virus (EBV). Moreover, TCRVβ21.3$^+$ T cells were identified in individuals with MIS-C that were specific to the EBV antigen EBNA2, highlighting a link between T cell dysfunction, EBV reactivation and hyperinflammatory conditions.

Serum levels of TGFβ1 were increased in children with acute MIS-C in comparison to children without any MIS-C symptoms 6 weeks after SARS-CoV-2 infection. The high serum levels of TGFβ1 in MIS-C correlated with its biological activity, as single-cell analysis of PBMCs indicated upregulation of TGFβ-induced genes in T cells and downregulation of antigen-presentation genes in monocytes and, to a lesser extent, in B cells — a phenomenon previously linked to TGFβ signalling. Indeed, in vitro assays with T cells and antigen-presenting cells from donors with acute MIS-C or with PBMCs from healthy donors that had been conditioned with MIS-C sera showed a TGFβ-dependent defect in T cell reactivity.

Despite this compromised reactivity, the T cell compartment of individuals with MIS-C was characterized by increased proliferation of recently-activated CD38$^+$ HLA-DR$^+$ T cells, and particularly the expansion of the TCRVβ21.3$^+$ T cell subset. To identify the antigenic drivers of this T cell expansion, the authors generated T cell receptor (TCR) libraries using T cells from healthy donors that recognized antigens of EBV, cytomegalovirus, adenoviruses, measles, or SARS-CoV2. TCRα chain analyses co-clustered TCRVβ21.3$^+$ TCRs of children with MIS-C with EBV-specific TCRs. Moreover, a subset of these TCRVβ21.3$^+$ T cells were specific to EBNA2 and displayed cytotoxic activity against EBV-transformed B cells.

As TGFβ suppressed T cell reactivity in children with MIS-C and has been previously shown to also induce the EBV lytic cycle, the authors next examined these individuals for signs of EBV reactivation. Indeed, they identified high titres of EBV-specific antibodies and increased EBV gene transcription in B cells and plasmablasts from children with MIS-C. Based on these findings, Mir-Farzin Mashreghi, co-corresponding author of the study, notes that "the combination of high serum levels of TGFβ1, EBV-specific antibodies and TCRVβ21.3$^+$ T cells might be a useful diagnostic tool to distinguish MIS-C from other hyperinflammatory conditions, such as Kawasaki disease". "SARS-CoV2-mediated activation of TGFβ and the associated EBV reactivation emerges as a temporary-acquired mechanism of hyperinflammation that might also apply to other diseases", adds Carl Christoph Goetzke, first author of the study. "Although MIS-C incidence has decreased, we expect that this condition will continue to affect children, especially those exposed to SARS-CoV-2 for the first time, potentially following a two-hit model", adds Mashreghi, highlighting plans "to continue this research to further understand the links between EBV reactivation and hyperinflammation or autoimmunity and to develop an EBNA2-based vaccine that strengthens T cell-mediated surveillance against EBV infection or reactivation".

Maria Papatriantafyllou

Original article: Goetzke, C. C. et al. TGFβ links EBV to multisystem inflammatory syndrome in children. *Nature* https://doi.org/10.1038/s41586-025-08697-6 (2025)

Therapy

Sex and gender matter for TNF inhibitor therapy in RA

Considering sex and gender differences when evaluating treatment response in rheumatic diseases is an important area of research. Studies on sex and gender differences in the treatment of rheumatoid arthritis (RA) are conflicting. Findings from a Danish cohort study (DANBIO registry) report differences between men and women in their response to TNF inhibitors.

This study included 7,789 individuals with RA (75% of whom were women) with similar disease activity at baseline. Response to treatment was measured 4 and 12 months after the initiation of anti-TNF therapy; the primary outcome was the proportion of patients achieving EULAR good response at the indicated timepoints.

After 12 months, men had a greater decrease in DAS28-CRP score than women, and the chance of achieving EULAR good response was 14% lower for women than for men. In addition, women ≤50 years of age were more likely than men of a similar age to terminate treatment owing to adverse effects.

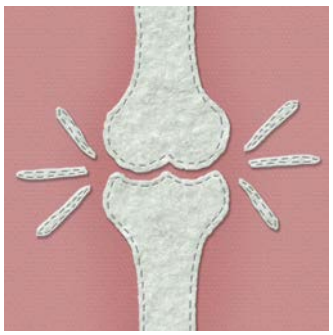
The mechanisms underlying the differences between men and women with RA in their response to treatment, whether with TNF inhibitors or other therapies, are unclear and require further study.

Holly Webster

Original article: Lauridsen, K. B. et al. Sex differences in treatment response in patients with rheumatoid arthritis treated with tumour necrosis factor inhibitor: a cohort study from the DANBIO registry. *Scand. J. Rheumatol.* https://doi.org/10.1080/03009742.2025.2471713 (2025)

Rheumatoid arthritis

CXCL7 promotes bone erosion in RA



Bone destruction is a hallmark feature of rheumatoid arthritis (RA), and individuals with anti-citrullinated protein antibody (ACPA)-positive RA tend to experience worse bone erosion than those with ACPA-negative RA. Chemokines, such as CXCL7, regulate bone erosion and a study has now provided insights into the role of CXCL7, which is highly expressed in the RA synovium, in bone destruction in RA.

Wang et al. first assessed the concentration of CXCL7 in serum from individuals with RA and healthy individuals. The concentration of CXCL7 was higher in plasma from individuals with ACPA-positive RA than those with ACPA-negative RA or healthy individuals. In addition, CXCL7 concentration correlated with bone erosion and disease activity in individuals with RA.

To determine whether CXCL7 alters osteolastogenesis, the authors isolated $CD14^+$ monocytes from the blood of individuals with RA and cultured these cells with CXCL7. The function of these cells was then tested using a bone resorption assay. CXCL7-treated $CD14^+$ monocytes enhanced osteoclast differentiation and promoted bone erosion. In addition, these monocytes had increased transcription of *RANK*, which encodes a receptor that binds to RANK ligand and stimulates osteolastogenesis. Further experiments using the RAW264.7 cell line (commonly used in osteolastogenesis research) demonstrated that the ERK and NFATc1 signalling pathways were required for CXCL7-induced osteolastogenesis.

In mice with collagen-induced arthritis (CIA), levels of plasma CXCL7 were increased when compared with control mice. Injecting CIA mice with a CXCL7-neutralizing antibody improved disease by reducing inflammation and osteoclast differentiation. Micro-CT scanning of the joints of these mice showed that blocking CXCL7 reduced bone erosion.

Together, this study provides important insights into the role of CXCL7 in RA pathogenesis. "Measuring CXCL7 levels might help to stratify high-risk patients with ACPA-positive RA for early, aggressive intervention. Our findings position CXCL7 as both a biomarker and a therapeutic target for rheumatoid arthritis", comments Jinxia Zhao, one of the corresponding authors of this study.

Holly Webster

Original article: Wang, X. et al. CXCL7 enhances RANKL-induced osteoclastogenesis via the activation of ERK/NFATc1 signaling pathway in inflammatory arthritis. *Arthritis. Res. Ther.* **27**, 34 (2025)

Osteoarthritis

Profiling synovial tissue reveals OA subgroups

In a new study, detailed analysis of human synovial tissue led to the identification of two types of knee osteoarthritis (OA) that are associated with distinct populations of synovial fibroblasts and macrophages. The findings also implicate a particular subset of synovial fibroblasts in the resolution of knee OA synovitis.

The study involved synovial tissue samples from 36 patients with knee OA undergoing primary total knee arthroplasty, and 5 patients undergoing knee arthroscopy 1 year after anterior cruciate ligament reconstruction. Through bulk RNA sequencing, the samples were classified into two subgroups, termed 'inflammatory' and 'fibrotic'. Notably, all samples obtained from the patients undergoing arthroscopy were classified as fibrotic.

In the inflammatory subgroup of OA, synovial tissue was characterized by increased expression of pro-inflammatory cytokines and chemokines and an increased frequency of infiltration of inflammatory cells into the synovium. By contrast, synovial tissue in the fibrotic subgroup was marked by increased expression of fibroblast growth factors and bone morphogenic proteins and the presence of collagen fibres on histological evaluation. Patients in the inflammatory subgroup reported worse pain scores than those in the fibrotic subgroup.

The researchers then used single-cell RNA sequencing to investigate synovial cell profiles in samples from representative patients in each subgroup. Consistent with previous studies, they identified three distinct subsets of synovial fibroblasts ($CD34^{lo}THY1^{lo}$, $CD34^{lo}THY1^{hi}$ and $CD34^{hi}$) and two distinct subsets of macrophages ($MERTK^{hi}CD206^{hi}$ and $MERTK^{lo}CD206^{lo}$). $CD34^{hi}$ fibroblasts, which had abundant expression of genes associated with fibrosis and angiogenesis, predominated in the fibrotic-type samples, whereas $MERTK^{lo}CD206^{lo}$ macrophages, which were marked by the expression of chemokines associated with leukocyte recruitment and activation, were more prominent in the inflammatory-type samples.

Ligand–receptor analyses suggested that $CD34^{hi}$ fibroblasts interacted most actively with mural and endothelial cells, and that $CD34^{lo}THY1^{lo}$ and $CD34^{lo}THY1^{hi}$ fibroblasts interacted mostly with synovial immune cells such as T cells, dendritic cells and macrophages. Among the $CD34^{hi}$ fibroblasts, a subset of $CD34^{hi}CD70^{hi}$ cells was identified that potentially suppress synovitis by promoting the proliferation of regulatory T cells.

The researchers suggest that knowledge of how synovial cell subsets are regulated in OA could lead to the development of novel therapeutics. "Early synovitis in knee OA has been reported as a risk factor for OA progression, so we are planning to identify a group of patients in whom OA progression can be prevented by treating synovitis," notes Junya Miyahara, first author of the paper reporting the results. "We are aiming to develop a non-surgical treatment that relieves clinical symptoms of OA by a mechanism different from NSAIDs and corticosteroids."

Sarah Onuora

Original article: Miyahara, J. et al. $CD34^{hi}$ subset of synovial fibroblasts contributes to fibrotic phenotype of human knee osteoarthritis. *JCI Insight* **10**, e183690 (2025)

Neuroimmunology

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Macrophages hit a nerve in painful joint venture

Oumaima Ben Brahim & Stefan Uderhardt

Hasegawa et al. reveal how synovial joints detect systemic inflammation through specialized fenestrated blood vessels at the synovial periphery. Three distinct macrophage populations and nociceptor neurons form a sentinel unit around these vessels, coordinating immune responses and pain signalling through interleukin-1β (IL-1β) and calcitonin gene-related peptide (CGRP) signalling.

Refers to Hasegawa, T. et al. Macrophages and nociceptor neurons form a sentinel unit around fenestrated capillaries to defend the synovium from circulating immune challenge. *Nat. Immunol.* **25**, 2270–2283 (2024).

Joint pain is a common manifestation of systemic diseases ranging from infections to autoimmune conditions, yet the mechanisms by which joints detect and respond to blood-borne threats have remained elusive. A new study by Hasegawa et al., published in *Nature Immunology*[1], reveals an intricate immunosurveillance system in joints that helps explain joint pain in disease and potentially opens new therapeutic avenues for joint pain and inflammation.

Three-dimensional tissue-microscopy techniques enabled the authors of this study to visualize entire synovial tissues surgically isolated from mouse knees, and revealed previously unknown structural features of joint immunology. Fenestrated capillaries positive for the endothelial marker PV1 were identified at the periphery of the synovium, particularly at the interface between the lining and sublining layers of the synovial membrane.

Fenestrated vessels have increased permeability and enable exchange between blood and tissues, and are typically found in organs heavily involved in systemic exchange, such as the liver, kidneys and intestines[2]. At first glance, the presence of fenestrated vessels in joints seems counterintuitive and potentially dangerous, making joints vulnerable to circulating threats. However, fenestration enables immunocomplexes and other molecules to exit the bloodstream and enter the joint tissue[3]. The positioning of three distinct types of tissue-resident macrophages and pain-sensing neurons (nociceptors) around these vessels seems to form an intricate defence mechanism that acts as a 'blood–joint barrier'[1] (Fig. 1). This surveillance system was found to operate through an orchestrated sequence of cellular interactions. Specialized LYVE1$^+$CX3CR1$^+$ tissue-resident macrophages were found to detect immunocomplexes entering through the fenestrated capillaries and respond by producing IL-1β. IL-1β in turn activates nearby nociceptors to release calcitonin gene-related peptide (CGRP). CGRP prompts other types of macrophages to form protective clusters around the vessels, creating a physical barrier against further threats. This cellular arrangement bears interesting similarities to immunosurveillance systems in other organs, particularly the kidneys, where specialized resident macrophages monitor peritubular capillaries for immunocomplexes[3].

The positioning of such a surveillance system at the periphery of the synovium is particularly noteworthy because this is the joint site first affected in rheumatoid arthritis[4]. Thus, whereas the blood–joint barrier normally helps protect joints, it might also contribute to pathology when dysregulated, which might potentially explain why joints are particularly susceptible to autoimmune attack. The authors demonstrate that LYVE1$^+$CX3CR1$^+$ macrophages express the inhibitory Fcγ receptor FcγRIIb. An absence of FcγRIIb leads to increased recruitment of neutrophils into the synovium, suggestive of a role in regulating joint inflammation[5]. These findings echo clinical observations showing increased joint damage in patients with rheumatoid arthritis who carry *FCGR2B* polymorphisms that impair receptor function[6]. Thus, therapeutically modulating FcγR function might have the potential to prevent joint damage in autoimmune diseases.

The results of the study might help to better elucidate several clinical observations that have long puzzled physicians. For example, they provide a mechanistic explanation for why joint pain is often associated with systemic infections or autoimmune diseases, even when the primary disease process occurs elsewhere in the body: circulating immunocomplexes enter the tissue through the fenestrated capillaries, where macrophages and nociceptors initiate joint pain signals.

From a therapeutic perspective, the finding that macrophage-derived IL-1β triggers joint pain through nociceptor activation suggests that targeting this pathway might provide focused pain relief without broadly compromising immune function. This is supported by previous studies showing that IL-1β has a role in inflammatory pain, both indirectly, via the production of inflammatory mediators[7], and via direct activation of nociceptors[8]. The observation that CGRP signalling influences macrophage responses provides a mechanistic explanation for reports of joint symptoms in patients receiving CGRP-targeting therapies for migraine. This bidirectional neuro–immune interaction suggests that although CGRP antagonism might benefit migraine, it might potentially alter protective macrophage responses in joints[9]. This aligns with growing evidence of a complex role for neuropeptides in immunoregulation and the broader concept of neuro–immune interactions in tissue homeostasis and inflammation[10].

From an evolutionary perspective, this surveillance system may serve dual purposes that benefit both individual survival and species protection. Joint pain and its consequent reduced mobility help conserve energy for mounting immune responses. Additionally, limited movement, general fatigue and social distancing could reduce disease transmission within populations — a behavioural adaptation known as 'sickness behaviour' that might have provided selective advantages by containing the spread of communicable diseases[11].

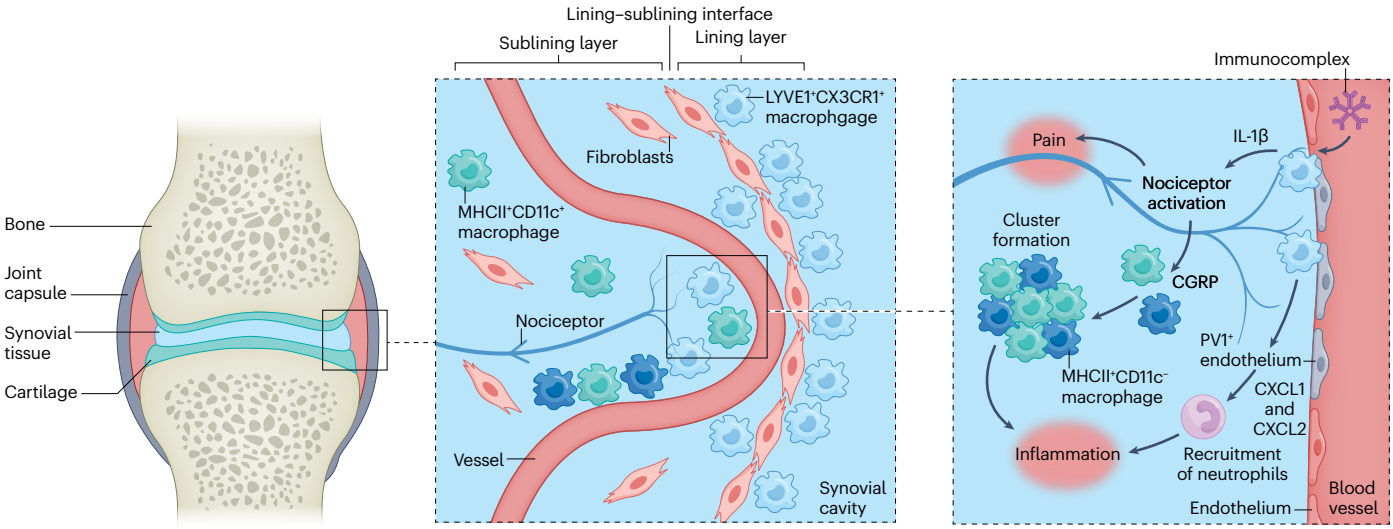

Fig. 1 | A synovial surveillance system. Synovial tissue macrophages recognize circulating immunocomplexes via fenestrated capillaries; this activates nociceptors and triggers local inflammatory tissue responses. CXCL1 and CXCL2, CC chemokine (C-X-C motif) ligands 1 and 2; CGRP, calcitonin gene-related peptide; IL-1β, interleukin-1β. Initial draft of Fig. 1 created using BioRender.

The study by Hasegawa et al.[1] reveals synovial joints as active participants in systemic immunosurveillance through an intricate cellular network. These findings contribute to understanding of the interaction between joints and systemic inflammation, in that the strategic arrangement of fenestrated vessels, macrophages and nociceptors creates an early warning system that protects joint integrity while contributing to host defence through pain-induced behavioural changes (Fig. 1).

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Competing interests

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Inflammatory arthritis

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Treating inflammatory arthritis in individuals with concomitant cancer

Maria E. Suarez-Almazor

Treating people with inflammatory arthritis and cancer is challenging given concerns around suppressing anti-tumour immunity. Targeted therapies, such as TNF inhibitors, can be safely used in patients with cancer who are in remission, but whether these treatments are safe for individuals with newly diagnosed or active cancer remains unclear.

Refers to Sebbag, E. et al. 2024 EULAR points to consider on the initiation of targeted therapies in patients with inflammatory arthritis and a history of cancer. *Ann. Rheum. Dis.* https://doi.org/10.1136/ard-2024-225982 (2024).

One in three people will develop cancer in their lifetime[1]. As such, many patients with inflammatory arthritis will have concomitant cancer at some point during their disease journey. Given the role of anti-tumour immunity in controlling cancer progression, there have been concerns about treating people with inflammatory arthritis and cancer with biologic and targeted synthetic DMARDs (bDMARDs and tsDMARDs). Past recommendations from professional societies are vague, offering general statements rather than specific guidance for different scenarios. Given the uncertainty surrounding decision-making for the treatment of these individuals, EULAR convened a task force to develop 'points to consider' to assist rheumatologists in decision-making when treating individuals with inflammatory arthritis (including rheumatoid arthritis (RA), psoriatic arthritis and spondyloarthritis) and a history of cancer[2].

To inform this guidance, the task force conducted a systematic literature review, which included 15 studies that evaluated cancer recurrence in patients with a history of cancer and inflammatory arthritis, inflammatory bowel disease or inflammatory skin disease who had been treated with any targeted therapy[3], Most of these studies included individuals with RA, and TNF inhibitors were the most frequently used targeted treatment. The pooled hazard ratio for cancer recurrence was 0.92 (95% confidence interval (CI) 0.74–1.15) when comparing patients receiving bDMARDs or tsDMARDs with those receiving conventional DMARDs. These findings suggest that targeted therapies are not associated with an increased risk of cancer recurrence; however, these studies mostly included patients who had been in remission for several years.

The task force proposed overarching principles to oversee this guidance. These overarching principles highlight the need to promptly treat patients to control inflammatory disease activity, as withholding treatment leads to poor functional outcomes. They also emphasize the need to consider the characteristics of individual patients and their medical history when determining the risk of cancer recurrence. Although this concept is crucial, there is limited evidence that supports the use of individual cancer characteristics (including cancer type, cancer stage, expected response to cancer treatment and time from cancer diagnosis) as a way to determine whether targeted therapies are safe to use. Despite an effort to stratify the analysis in the review according to cancer type and to the time from cancer diagnosis to initiation of targeted therapy, most studies included several cancers, and patients were primarily long-term survivors in remission, with a median time from cancer diagnosis to initiation of bDMARDs or tsDMARDs of 4 years. For example, a patient with a remote history of stage 1 breast cancer will have a very different risk of recurrence from a patient with newly diagnosed stage 3 pancreatic cancer. Thus, whether targeted therapies are detrimental for individuals with newly diagnosed cancer, more advanced disease, or in those with aggressive or more difficult to treat malignancies remains unclear.

Another of the overarching principles highlights the importance of rheumatologists involving patients in the treatment decision-making process. To optimize outcomes, a clear understanding of the potential harms and benefits is needed, especially in the face of uncertainty on how treating inflammatory arthritis could alter cancer prognosis. Shared decision-making is essential to achieve a decision that is concordant with patient values, preferences and tolerance to risk. Many factors can affect patient preferences, such as quality versus quantity of life, age, other comorbidities, family and social environment or cultural beliefs. Some people are risk averse and might not be willing to receive treatment if there is uncertainty about potential harms, whereas for others, symptom control and being able to engage in day-to-day activities is more important than a potential risk of recurrence.

The task force proposed eight 'points to consider' (Box 1), with specific guidance that improves previous disease management recommendations from professional societies. They recommend the use of TNF inhibitors for patients with solid malignancies (other than melanoma) and in cancer remission. The evidence is too scarce to provide guidance for other drugs. Caution on the use of abatacept and Janus kinase (JAK) inhibitors is based on indirect evidence of increased incidence of new cancers in patients receiving these drugs. Data on rituximab and IL-6 receptor inhibitors are largely lacking, but there are no major safety signals. Finally, there is no information on other biologic agents used for spondyloarthritis (IL-17, IL-23 or IL-12–IL-23 inhibitors), hence TNF inhibitors are preferred for these patients.

There are other gaps in the available evidence that limit the scope of this guidance. Most evidence pertains to patients who have been in cancer remission for many years and, in general, rheumatologists are not as concerned about treating patients that do not have evidence of active cancer. What is more concerning is whether patients with newly diagnosed cancer can be safely treated with targeted therapies; very few studies have addressed treatment safety in this population. Two of our studies from 2024 and 2025 evaluated patients with RA who had been newly diagnosed with one of the most common cancers

Box 1 | Summary of EULAR guidance for the treatment of patients with inflammatory arthritis and cancer

1. Patients with active inflammatory arthritis and a history of cancer should be treated without delay.
2. Risk–benefit considerations should balance the harm of not treating inflammatory arthritis with the potential for cancer progression when using targeted DMARDs.
3. Multidisciplinary care is necessary for the management of patients with inflammatory arthritis and cancer.
4. Patients with inflammatory arthritis and a history of cancer in remission can receive targeted anti-rheumatic treatment.
5. JAK inhibitors and abatacept should only be used if there are no other alternatives.
6. TNF inhibitors are preferred in patients with solid malignancies, other than melanoma, over other targeted therapies.
7. B cell-depleting therapies, such as rituximab, might be preferable in patients with a history of lymphoma.
8. The decision to use targeted anti-rheumatic therapies should be a shared decision between the patient, their rheumatologist and their oncologist.

(breast, colorectal, lung or prostate) and treated with TNF inhibitors in the first 3 years after cancer diagnosis[4,5]. There were no differences in overall or cancer-specific survival in patients treated with TNF inhibitors compared with those treated with conventional DMARDs or no DMARDs. Stage at diagnosis is also an important variable; localized tumours are less likely to recur than more invasive regional ones, but this variable is not considered in most studies.

Rheumatologists are also concerned about the use of targeted therapies in patients with active cancer, or in those with metastatic disease. Advances in oncological treatments mean that many patients live for years with stable metastatic disease, and cancer is increasingly being considered a chronic disease[1]. The safety of targeted therapy in this population has not been established. Similarly, there is almost no data on the use of targeted therapies in patients with advanced cancer and short life expectancy. In this population, considering quality versus quantity of life and patient preferences is essential. Finally, some cancers, such as melanoma, are highly immunogenic, whereas others are not; the potential harm of targeted therapies could vary according to how immunogenic a cancer might be. Moreover, the effects of treating patients with inflammatory arthritis who are receiving cancer immunotherapy, such as immune checkpoint blockade, with bDMARDs and tsDMARDs are largely unknown.

The EULAR guidance is a welcome step in the right direction to aid health-care providers and patients in decision-making when considering the use of targeted therapy in people with inflammatory arthritis and a history of cancer. It also highlights gaps in evidence that should be addressed in future studies.

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Competing interests

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nature reviews rheumatology

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Review article

Check for updates

The causal role of brain circuits in osteoarthritis pain

Joana Barroso[1,2], Paulo Branco[1] ✉ & A. Vania Apkarian[1,2,3] ✉

Abstract

Osteoarthritis (OA) is a leading cause of chronic pain worldwide, resulting in substantial disability and placing a substantial burden on patients and society. The hallmark symptom of OA is joint pain. Despite extensive research, new treatments for OA pain remain limited, partly owing to a lack of understanding of underlying pain mechanisms. For a long time, OA pain was seen as a reflection of nociceptive activity at the joint level, and the brain has been viewed as a passive recipient of such information. In this Review, we challenge these concepts and discuss how, over time, the activation of peripheral nociceptors leads to adaptations in the brain that dictate the properties and experience of OA pain. These adaptations are further influenced by the inherent properties of the brain. We review general concepts that distinguish pain from nociception, present evidence on the incongruity between joint injury and experience of OA pain, and review brain circuits that are crucial in the perception of OA pain. Finally, we propose a model that integrates nociception, spinal-cord mechanisms, and central nervous system dynamics, each contributing uniquely to pain perception. This framework has the potential to inform the development of personalized treatment strategies.

Sections

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Key points

- Osteoarthritis (OA) commonly leads to chronic pain, and as OA pain becomes clinically apparent over time, brain adaptations to the disease have the potential to shape the characteristics and experience of pain.
- The brain's role in OA pain is shaped not only by sensory pathways but also by cognitive appraisal, emotional processing and descending modulation, all of which interact to influence pain perception.
- Models that integrate the diverse peripheral and central mechanisms of OA pain can uncover key processes, identify therapeutic targets and establish probabilistic approaches for treatment outcomes, potentially paving the way for personalized decision-making.
- The relative contributions of nociceptive activity, spinal-cord sensitization and supraspinal modulation to OA pain — and to pain relief with treatments — remain to be fully identified, highlighting the need for further scientific efforts to measure their interactions.
- OA pain treatment might benefit from shifting its focus from primarily targeting the joint to incorporating both peripheral and central neuroplasticity-based interventions.

Introduction

Osteoarthritis (OA) is a chronic joint disorder characterized by abnormal structural changes in the joint and surrounding tissues. OA is traditionally defined as a disease of movable joints involving cellular stress and extracellular matrix degradation triggered by micro- and macro-injuries. These injuries prompt maladaptive repair responses, including activation of pro-inflammatory pathways, leading to abnormal joint tissue metabolism and subsequent structural and functional changes such as cartilage degradation, bone remodelling, osteophyte formation and joint inflammation, ultimately resulting in symptomatic disease — pain, stiffness, swelling and loss of normal joint function[1–3]. There is extensive literature regarding the biology of the OA joint[2–4], and the topic will not be covered in this review.

The primary manifestation of OA, and indeed the hallmark symptom driving patients to seek medical attention, is joint pain[5]. Whereas the disease might silently progress over months or years, it becomes clinically evident once pain emerges. Pain, as defined by the International Association for the Study of Pain, is an "unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"[6]. This definition highlights pain as a complex, multidimensional experience encompassing both sensory and emotional (affective) components[7,8]. Acute pain typically relates to immediate tissue damage or injury, whereas chronic pain, as defined by the International Association for the Study of Pain, persists beyond 3 months[9] and is increasingly recognized as involving unique neuroplastic changes, establishing itself as a distinct disease process. Unlike acute pain, which often resolves as the injury heals, chronic pain might result from an unresolved injury, keeping all components of the pain experience active and evolving. Alternatively, chronic pain might develop as a maladaptive response to an initial injury, signalling underlying mechanisms that differ substantially from those in acute pain.

In this review, we examine the evidence and the underlying mechanistic concepts of pain in OA, which, in our view, involves three steps. First, prolonged mechanical injury, joint remodelling and persistent inflammation trigger the initial activation of peripheral nociceptors in the joint. Second, over time, and when OA pain is clinically identified, brain adaptations in response to injury modulate the properties and experience of OA pain; these adaptations are also influenced by inherent properties of the brain. Third, in a substantial subgroup of patients with OA, these brain adaptations maintain the experience of pain after the nociceptive contribution originating from the diseased joint is reduced, for example, after total joint replacement (TJR). We, thus, review general concepts that distinguish pain from nociception, discuss evidence on the incongruity between joint injury properties and the OA pain experience, and review brain circuits that are crucial for the perception of OA pain. Finally, we propose a model that integrates nociception, spinal-cord mechanisms, and central nervous system dynamics as components of OA pain. This framework allows for the exploration of various factors that influence pain perception in OA, recognizing the heterogeneity among patients and potentially guiding personalized interventions.

General concepts distinguishing pain and nociception

In a series of papers, we have formulated concepts that differentiate pain and nociception[10–12].

Whereas nociceptors can drive the conscious perception of pain, prompting protective behavioural changes, we argue that nociception operates on a continuum, and that nociceptive activity without the conscious experience of pain has a crucial role in guiding unconscious behavioural actions, allowing individuals to act in ways that minimize the risk of pain or injury. In this context, pain would emerge when nociceptive mechanisms are overwhelmed, signalling that the protective role of nociceptors has been compromised, and indicating a risk of or occurrence of tissue damage[12]. Thus, pain is necessarily a conscious state tightly linked to negative affect; both properties ensure attending to the event and learning and modifying the repertoires of behaviours that are usually driven by nociception. This view contrasts with Sherrington's early twentieth-century definition of nociception as the neural correlate of pain perception[13], a concept that remains prevalent, particularly in OA research, in which the focus on nociception and the joint injury-associated peripheral nociceptive activity overlooks the multifaceted nature of OA pain mechanisms[14].

A corollary to the distinction between nociception and pain is that negative emotions and moods alone should not be equated with pain, just as injury and nociceptive signals do not inevitably lead to pain. Both nociceptive activity and emotional responses contribute to the pain experience[11,15]. From this perspective, chronic pain can be viewed as a spectrum of persistent conditions, characterized by an amplification of the nociceptive afferent system at peripheral and spinal-cord levels, an increase in the affective impact of nociceptive input through central limbic pathways, or (most likely) a combination of the two[12,16]. These mechanisms might eventually maintain the chronic-pain brain state. It is important to emphasize that the modulation of nociceptive inputs at spinal, limbic and cortical levels is fundamental to endowing pain its subjectivity (both acute and chronic) and its multidimensionality, as well as defining its attentional, emotional and motivational attributes[15]. Furthermore, chronic pain evolves into a heightened state of learning and becomes pathological as its relationship with the environment degenerates[17,18]. The disconnection between perceived pain and environmental cues, in turn, renders the pain as belonging to the self, enhancing negative affect and diminishing cognitive control[16,19,20].

In this review, we pinpoint evidence of the mechanisms that link these ideas to OA pain.

Discrepancy between joint damage and pain in osteoarthritis

Biomarkers of osteoarthritis joint damage

The incongruity between OA injury site structural changes, physical impairment and levels of self-reported pain has been repeatedly reported[21,22]. Remarkably, in radiographically identified knee degeneration, only around 50% of the affected individuals report knee pain, and conversely, across 1,004 patients with knee pain, only 15% had radiographically identified abnormalities[22]. Another study showed that even among patients with severe knee radiographic OA, around 6–30% do not report knee pain[23]. Meta-analytical evidence indicates a greater likelihood of OA pain in the presence of MRI knee lesions (odds ratio 2.4 to 10.5), yet the level of such evidence is rated as moderate or conflicting[24]. In general, the evidence linking structural changes observed through imaging of the joint level to OA pain is weak and variable (Fig. 1). Structural changes seen in joints via imaging do not provide evidence of peripheral nociceptive activity, and the degree of joint injury is insufficient to explain OA pain. Likewise, although inflammation is thought to be an important factor in the development of OA pain, evidence supporting this association is scarce. Some studies link serum inflammatory biomarkers to pain intensity in OA[25–27], and increased expression levels of interleukin-1β (IL-1β)[28], the chemokine CCL2 (ref. 29), or C-reactive protein (CRP)[27] have been associated with pain in OA (Fig. 1). Although emerging molecular biomarkers, including inflammatory biomarkers, metabolites and genetic components, continue to be studied[30,31], there are no reliable molecular biomarkers firmly linking OA disease with OA pain.

Treatment-response phenotypes

Substantial interindividual variability exists in the response to treatments for OA pain, encompassing both pharmacological and non-pharmacological interventions[32] (Fig. 1). This variability extends across pharmacological treatments, such as NSAIDs, paracetamol and duloxetine, as well as non-pharmacological modalities, such as physical therapy, therapeutic exercise and intra-articular interventions[33]. Moreover, there are no established clinical predictors of response to these agents. Therefore, researchers have sought to define clinical phenotypes of OA[34,35], using multiple biomarkers: radiographic characteristics; clinical symptoms; metabolic and hormonal factors; psychological traits; demographic and socio-economic factors, applying outcome-guided[36–39], supervised[40] and unsupervised[41,42] data-driven methods. Importantly, measures of quantitative sensory testing that probe for neurobiological components have been integrated into the phenotyping of OA[42,43]. However, although the phenotyping approach might help to define clinical subgroups of patients with OA, its heavy focus on clinical characteristics often overlooks supraspinal mechanisms of pain, including brain structural and functional indices, as well as the affective–motivational and cognitive–evaluative components of pain.

Placebo effects, expectations and learning

The subjective and evaluative nature of OA pain beyond nociception is perhaps best highlighted by the prevalence and large effect size of placebo effects observed across OA clinical trials[44] (Fig. 1). A meta-analysis of 193 trials showed an average placebo effect size of 0.51 (ref. 45), which is clinically significant and similar to the effect sizes observed with most treatments for OA pain[46,47]. Meta-analytical findings also show that about 75% of the analgesia observed in clinical trials of OA can be explained by the placebo effect[46], indicating that the added benefit of active treatment is minimal compared with placebo alone, even for some surgical procedures[48]. As placebos are inherently inert agents or treatments, placebo effects are expected to be primarily driven by brain responses to expectations and thus linked with learning mechanisms[44,49–53]. Placebo effects engage the brain's endogenous opioid system[44,54,55], control ascending levels and modulate the value of peripheral nociceptive activity[56,57] on the basis of expectations and past experiences. More generally, the placebo effects showcase how factors beyond the joint influence OA pain perception and, in this sense, pain perception can be considered an inference about the underlying state of the body and self. Indeed, a wealth of research has focused on this topic[58–60].

Chronic pain after joint replacement surgery

TJR surgery is a common procedure for treating severe OA of the knee and hip. The procedure is considered safe and effective, and has been recommended as a treatment option when pharmacological and conservative treatments do not provide adequate pain relief and functionality[5]. TJR is amongst the most frequently performed surgeries worldwide, and large increases in the demand for TJR are projected

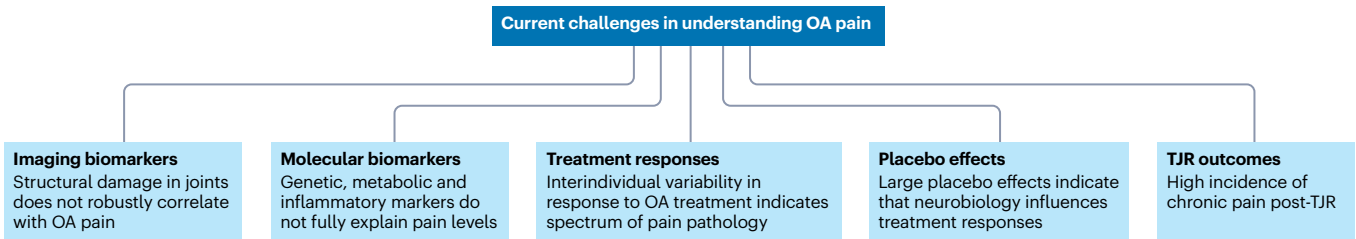


Fig. 1 | Current challenges in understanding mechanisms of pain in osteoarthritis. The lack of direct association between the magnitude of pain and pain progression in patients with osteoarthritis (OA) and imaging or molecular biomarkers of joint damage indicates that the subjective pain experience might be dissociated from the peripheral disease in OA. Structural changes in imaging do not provide evidence of peripheral nociceptive activity and the degree of joint injury is insufficient to explain OA pain. Although molecular biomarkers (inflammatory, metabolic, and genetic) continue to be studied, no reliable correlates have linked OA local disease with OA pain. The interindividual variability in responses to various classes of OA treatments highlights the heterogeneity of pain pathophysiology in OA, whereas the extensive placebo effects reported in trials of OA treatments underscore the potential contribution of neurobiological influences to OA pain experience. The prevalence of chronic pain after total joint replacement (TJR) therapy suggests that central neuroplastic changes have a role in pain perception even after the peripheral nociceptive insult is excluded. An improved understanding of how adaptive and maladaptive plasticity in brain circuits during the course of joint disease is essential for addressing gaps in OA pain treatment.

for the future[61,62]. Although TJR is seen as highly successful in alleviating pain and improving function, 7–23% of all patients with hip OA and 10–34% of patients with knee OA report moderate-to-severe pain between 3 months and 5 years after surgery[63]. Moreover, population surveys indicate that TJR outcomes might not be as positive as indicated in the literature: across more than 22,000 surveyed individuals, up to 40% were unsatisfied with their TJR outcome, and only 22% rated the outcome as 'excellent'[64–66]. Thus, the most successful procedure in relieving pain and improving function, TJR, which is also the costliest intervention and requires extended periods of rehabilitation, still leads to unsatisfactory outcomes in many patients (Fig. 1).

Multiple pre-operative clinical, demographic and psychological variables have been studied as possible risk factors for post-TJR pain. Presurgical pain intensity, pre-operative function, and psychological variables — including anxiety, depression, pain catastrophizing and coping strategies — are among the most frequently studied potential risk factors[67–70]. Female gender, younger age, medical co-morbidities, lower socio-economic status and increased rates of opioid use before surgery have also been proposed as individual risk factors for pain after TJR[68,71,72]. Post-operative risk factors for post-total knee replacement pain have also been studied; acute post-surgical pain, early post-operative functional outcomes, post-operative mood parameters, and opioid use after surgery have been linked with negative outcomes[73]. This evidence remains conflicting and assessed to be of low quality, with many of these associations being small in magnitude and thus of minimal biological interest[74].

Pain responses to acute noxious stimuli on the skin of patients with OA have been studied as predictors of post-TJR pain, as assessed using quantitative sensory testing measures, including pressure pain thresholds, sensitivity mapping, mechanical temporal summation and conditioned pain modulation[75]. These measures have been interpreted as clinical measures of central sensitization: for example, patients with knee OA and with strong hyperalgesia show high temporal pain summation after repetitive stimulation of the OA knee, whereas patients with widespread pain beyond the joint exhibit low pressure-pain thresholds[76]. The presence of such indicators before surgery has been linked to a worse outcome[77,78]. Moreover, pain intensity ratings to pinprick stimulation of the OA knee before surgery predict post-surgical pain, but account for only about 20% of pain variance 12 months after knee TJR[79]. However, other studies report non-significant associations between such indices and the persistence of OA pain[80].

Overall, an extensive list of clinical parameters has been tested as prognostic for post-TJR pain[68]. Their overall utility in clinical decision making requires more comprehensive studies. Below, we expound on the potential correspondence between such measures and brain-derived biomarkers of OA pain.

The brain in chronic pain

The role of the brain in OA pain has been somewhat less frequently studied than other pathophysiological aspects of OA, although the same cannot be said for other pain conditions. The contribution of brain circuits has been studied extensively in chronic low back pain, fibromyalgia, complex regional pain syndrome and neuropathic pain, both in animal models and in human studies[12,81,82]. There is now clear evidence that chronic pain is associated with large anatomical and functional changes in the brain[83–87]. Certain adaptations are common across chronic pain conditions, but each syndrome presents a unique pattern of brain reorganization[88]. This evidence highlights that the brain undergoes plastic changes in response to chronic pain, establishing a distinct state compared with acute pain[19,89], and also indicates that the various conditions might be accompanied by specific adaptive and maladaptive brain changes that define the details of the specific pain experiences. Overall, the pain neuroimaging field has unveiled three fundamental pillars of knowledge regarding the supraspinal processing of pain that could pave the way for rethinking OA pain. First, pain should not be viewed solely as directly dependent on the level of nociceptive input but rather as dependent on the processing mechanisms of such stimuli[90,91]. Second, pain perception reflects the cumulative peripheral and central adaptations associated with the burden of persistent or recurrent pain. Third, pain is also influenced by pre-existing brain properties and their unique interaction with nociceptive activity arising from a peripheral injury[12,19,92–94].

Given the multidimensional nature of pain[7], the brain has a central role in shaping various aspects of the pain experience, which can be broadly categorized into distinct domains (Fig. 2a). The classic neurobiological framework of pain[7,95] has proposed three core domains of pain experience: the sensory–discriminative domain, which encodes nociception, location, intensity and qualia of the nociceptive information; the affective–motivational domain, which supports the appraisal of nociception, motivates behavioural change and facilitates learning and avoidance through positive and negative reinforcement; and the cognitive–evaluative domain, which integrates sensory and affective information and directs attention to sensory stimuli. Expanding on this traditional view, we also discuss a fourth, now well-established domain: descending modulation, which enables the brain to modulate nociceptive signals by exerting control over spinal cord circuitry[96]. Below, we review findings from the OA literature that show how these systems are impacted by, and relate to, OA pain.

Brain adaptations in osteoarthritis

Nociception and the sensory–discriminative component of pain in osteoarthritis

The sensory–discriminative component of pain traditionally involves the ascending nociceptive spinothalamic pathway, including the lateral thalamus, the primary and secondary sensory cortices, the primary motor cortex and the posterior insula. Together, these brain regions are thought to provide information about the duration, sensation and location of the external stimulus. Although these circuitries are traditionally seen as solely interpreting incoming nociceptive signals, there is well-established evidence that chronic OA pain leads to plastic changes in these brain regions, underscoring the brain's dynamic involvement in OA pain.

The thalamus is perhaps the brain region best studied in OA along the nociceptive discriminative pathways. Neuroimaging studies comparing patients with chronic OA and healthy individuals report that patients with OA have lower grey matter density in the thalamus[97,98] and lower concentrations of N-acetyl aspartate, which is a putative marker of neural integrity[99,100]. Similarly, there is evidence of lower baseline cerebral blood flow in the thalamus in patients with chronic OA than in healthy individuals, suggesting that thalamic function is abnormal[101]. Abnormal activity in the thalamus might also reflect increases in neuronal activity caused by ongoing nociceptive signalling. Indeed, functional connectivity — that is, the synchronized activity that two or more brain regions exhibit over time as a result of the exchange of information between them — from the ventrolateral nucleus of the thalamus to the rest of the brain is increased in proportion to ongoing pain intensity in patients with OA[102]. Deliberate increases in pain in

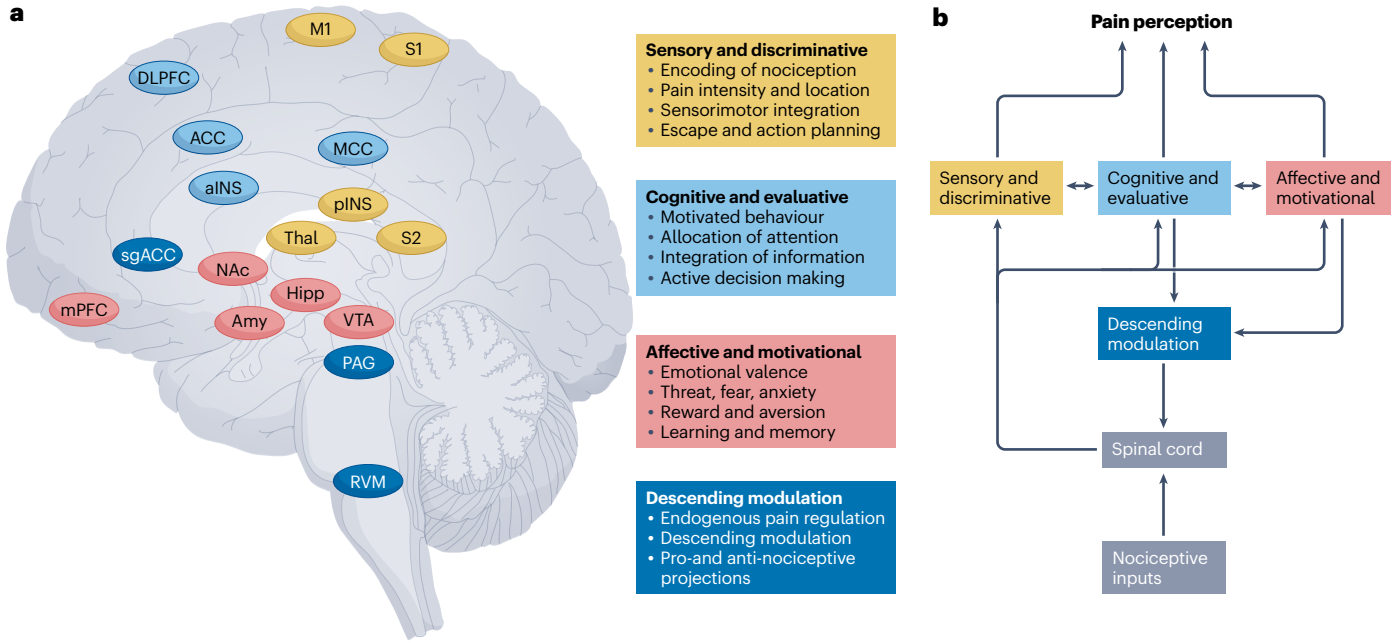


Fig. 2 | Brain circuits in osteoarthritis. a, Multiple brain circuits are associated with the experience of pain in osteoarthritis. They can be broadly summarized in four domains, as initially described by Casey[7] and expanded here. The sensory and discriminative (yellow) domain, including the primary motor cortex (M1), primary sensory cortex (S1), secondary sensory cortex (S2), thalamus (Thal) and posterior insula (pINS), has been recognized for its role in relaying nociceptive information to the cortex, encoding pain location, qualia and generating adaptive motor responses to pain. The affective and motivational (red) domain, including the ventral tegmental area (VTA), the nucleus accumbens (NAc), the hippocampus (Hipp), the amygdala (Amy) and the medial prefrontal cortex (mPFC), provides an affective valuation to incoming nociceptive signals and provides the necessary signal for behavioural modification. The affective and motivational domain is involved in emotional responses to pain, aversive and reward signalling, and learning and consolidation of pain experiences in memory. The cognitive and evaluative (light blue) system, including the anterior insula (aINS), the anterior and medial cingulate cortex (ACC and MCC, respectively), and the dorsolateral prefrontal cortex (DLPFC), plays a key role in the integration of sensory and affective aspects of pain, as well as allocating attentional resources to attend to painful stimuli, or inhibit these stimuli depending on their behavioural relevance and salience. Finally, the descending modulation (dark blue) system consists of several regions of the endogenous opioidergic system, namely the subgenual ACC (sgACC), the periaqueductal grey area (PAG) and the rostroventral medulla (RVM), and exerts control over incoming nociceptive stimuli through direct projections to spinal circuitry. The descending modulation system can condition (or facilitate) the ability of dorsal horn neurons to convey nociceptive information to the brain. **b**, These four systems interact to shape pain perception by imbuing nociceptive inputs with affective and sensory dimensions, directing attentional and cognitive resources to these stimuli, and enabling the brain to regulate nociception through descending modulatory pathways. Some brain regions might have multiple roles in pain perception, which have been simplified here for brevity. Nevertheless, all four brain domains contribute to the subjective pain experience.

these patients – for instance, after they perform painful manoeuvres – result in increases in functional connectivity between the thalamus and other brain regions implicated in pain[103]. Given the role of the thalamus as a relay of nociception, these abnormalities are likely to act as a first step in supraspinal amplification of the nociceptive barrage as the disease progresses, and this amplified signal is then propagated to other systems.

Nociceptive information arriving at the lateral nuclei of the thalamus is further relayed to the primary and secondary sensory cortices, where the brain processes sensory dimensions such as stimulus intensity, quality and location[104,105]. As with thalamic findings, patients with OA show atrophy of the primary sensory cortex[106], with lower volumes of the sensory cortex being associated with reduced pain thresholds[106], suggesting increased amplification of nociceptive signals in patients with OA compared with healthy individuals. Similarly, the primary motor cortex has also been shown to undergo plastic changes in OA, given the long periods of compromised mobility and related learned motor adaptations. Indeed, there is evidence of shrinkage of the primary motor cortex[97], increased spontaneous neural activity[107] and distortion of the motor homunculus in patients with OA[108]. These changes are not necessarily pain specific and might instead reflect behavioural and motor adaptations over the course of the disease. However, the motor cortex was shown to include integrative hubs (that is, specialized regions responsible for integrating brain-wide information) spaced between motor representations that project to other pain-related areas[109], and these integrative hubs have been implicated in pain integration and anticipation. This potential role of the primary motor cortex in pain is consistent with evidence that motor cortex stimulation with transcranial direct current stimulation improves pain in patients with OA[110].

In summary, the sensory–discriminative system, traditionally associated with relaying nociception and processing the sensory dimensions of pain, shows substantial plasticity in chronic OA. Structural and functional changes in key regions such as the thalamus, sensory and motor cortex suggest that persistent nociceptive input leads to widespread neural reorganization. These adaptations might, in turn,

contribute to increased pain sensitivity and altered pain thresholds, heightening the pain experience.

Descending modulation of pain

The brain exerts control over spinal cord nociceptive processing through descending modulatory control systems. The two key regions within this system are the periaqueductal grey area (PAG), and the rostroventral medulla (RVM), two brain areas that have a key role in endogenous analgesia, including placebo-mediated analgesia, and a primary site of action for several classes of analgesic drugs, including opioids[111] (Fig. 2). The PAG projects to the RVM, which in turn projects to the dorsal horn of the spinal cord, allowing this system to modulate the activity of spinal nociceptive signals projecting cephalad[111,112]. Both the PAG and RVM receive inputs from several cortical and subcortical brain regions implicated in cognition, affect and stress — most notably the rostral and anterior cingulate cortex (ACC), the ventromedial prefrontal cortex (vmPFC), the hypothalamus, and the amygdala — and through these pathways, cognitive, evaluative and motivational brain systems can control spinal nociceptive signals. There is evidence that this system can be facilitatory or inhibitory[113], dynamically controlling ascending nociceptive pathways on the basis of behaviour, as well as cognitive and affective states. Preclinical evidence suggests that descending facilitation might be crucially related to central sensitization and widespread hyperalgesia[114,115], indicating that as pain becomes chronic, the balance shifts in favour of facilitation of transmission of nociceptive inputs to the brain[115,116].

Neuroimaging findings in patients with OA suggest an abnormal or compromised descending modulatory system. As compared with healthy individuals, patients with OA exhibit lower cerebral blood flow in the PAG[101] and increased activity as a response to punctate stimuli applied to the injured joint; this increased activity correlates with reported neuropathic pain qualities[98]. RVM and cingulate cortex responses to punctate stimuli have also been shown to be higher in patients with OA who have neuropathic pain qualities than in those lacking neuropathic pain, and, perhaps most importantly, this abnormal RVM activity predicts the development of chronic post-surgical pain after TJR[117]. As in this study abnormal RVM activity was captured before surgery, it does not reflect surgical consequences and, thus, implies that the brain is either pre-emptively sensitized to incoming nociceptive inputs, possibly due to a chronic state of nociceptive facilitation, or primed to amplify these signals.

A key role in the modulation of OA pain has also been attributed to brain regions that project directly to the PAG–RVM. For example, when the functional connectivity increases between the vmPFC and the PAG as a result of exercise therapy, patients with OA experience pain relief[118]. Other studies in OA associate increases in functional connectivity between cortical brain regions and descending modulatory systems with pain relief resulting from manipulating expectations[119,120], or learning pain-coping strategies[121]. Together, these findings suggest that sensory, affective and cognitive systems can exert control over nociception through descending modulation, and that this modulatory system is also likely to be impaired in a subset of patients with OA — particularly those showing local and secondary hyperalgesia and neuropathic pain qualities.

Pain affect and emotional learning in osteoarthritis pain

The limbic system is a set of interconnected brain structures involved in cognition, emotions and memory that work together to influence behaviour, learning and autonomic functions[122,123]. This system has been implicated in pain processing, although it is less frequently studied than sensory and discriminative brain systems. Among limbic brain regions, the mesocorticolimbic system — which integrates the mesolimbic system (the ventral tegmental area (VTA) and nucleus accumbens) and the corticolimbic system (amygdala, hippocampus and vmPFC) pathways (Fig. 2a) — has long been implicated in the pathogenesis of chronic pain[12]. The overall hypothesis, based on several clinical and preclinical findings[124–127], argues that as pain becomes chronic, there is substantial remodelling of synaptic strength of mesocorticolimbic circuits that code affective aspects of pain. As a consequence, incoming nociceptive inputs are interpreted as more salient or aversive in patients with chronic pain than in healthy individuals, and this impacts pain perception[128]. The mesocorticolimbic system further provides a learning signal that guides adaptive behaviour through aversive and reward signalling, thus priming the system to shift its behaviour to cope with pain. This plasticity is likely to be adaptive in the acute injury stage as it helps to protect from further tissue harm, but, if not reversed, might perpetuate the maladaptive chronic pain state[124–127].

As for other chronic pain conditions, the mesocorticolimbic system has also been shown to undergo plastic adaptations over OA progression. For instance, the nucleus accumbens[106], hippocampus[107,129] and amygdala[98,106,130] exhibit reduced grey matter volumes in patients with OA[131,132]. There are also functional deficits in some of these regions, particularly low cerebral blood flow in the amygdala and hippocampus[101], and increased spontaneous firing of hippocampus neurons[107]. The exact timeline of these changes is hard to assess given the slow and uncertain onset of OA disease. However, preclinical research in mouse models of OA has shown large-scale changes in functional connectivity from the PAG to the amygdala, the nucleus accumbens and the hippocampus within days of OA onset, particularly in female mice[133], suggesting that brain plasticity during the early stages of disease might contribute to the transition to chronic pain, as also reported for preclinical models of neuropathic pain[125]. Complementary preclinical studies also show large changes in functional connectivity from the left and right nucleus accumbens to the VTA and PAG[134], potentially highlighting how the mesolimbic system's control over descending modulatory systems may be altered in the chronic OA stage.

The vmPFC, a key structure of the mesocorticolimbic system, also shows a decrease in grey matter volume in OA[98]. Importantly, the vmPFC and its extended circuitry is massively engaged when patients are asked to track their own ongoing pain in real time, but not when they track responses to experimental painful stimuli[135]. These findings, thus, link vmPFC function to an affective evaluation of the patients' ongoing pain, rather than during experimentally induced acute pain.

The mesocorticolimbic system is also involved in nociception and pain. For instance, OA pain has been associated with increased activity in the amygdala[136], and both amygdala and hippocampal responses to experimental painful stimuli decrease after intake of NSAIDs[137], hinting that these structures might encode nociception. However, other studies suggest that although spontaneous activities of the amygdala and hippocampus do indeed correlate with ongoing pain-intensity ratings, this correlation seems to also involve self-reported anxiety[138], indicating that the role of these brain structures is more likely to be related to the processing of non-sensory aspects of pain, such as a motivational assessment of incoming nociceptive information, rather than with the processing of nociceptive signals. Indeed, amygdala responses to spontaneous knee pain in OA are larger than amygdala responses to experimentally induced — but intensity-matched — knee pain, suggesting that

the patient's intrinsic OA pain is evaluated differently by the brain[136]. Along the same line, mechanical, but not thermal, stimuli were associated with enhanced responses of neurons in the amygdala in rats after experimental induction of knee arthritis[139]. The lack of sensitization to thermal stimulus argues against a generalized pro-nociceptive state, and instead again points to a specific learned association between pressure and pain, an association that more accurately mirrors the pain experience in knee arthritis[139,140]. Optogenetic stimulation of central amygdala neurons has also been shown to regulate dorsal horn neurons and enhance their response to painful stimuli in both mice with and without arthritis[141]. This underscores the causal role of the amygdala in pain and its capacity to modulate pain via descending modulatory circuits.

In patients with OA, both the amygdala and hippocampus increase their activity when patients are actively using pain-coping skills during painful stimulation[121]. Information exchange from the nucleus accumbens to the ventromedial and dorsolateral prefrontal cortex is also increased on the basis of modulation of treatment expectations[142], and increased functional connectivity from the VTA to the medial PFC has also been shown as a consequence of pain relief with exercise[118]. These findings showcase how manipulations that seek to reduce nociceptive input or change the way in which an individual evaluates and copes with pain are modulated by properties of the mesocorticolimbic system.

In summary, the mesocorticolimbic system has a crucial role in the affective and motivational dimensions of chronic pain in OA. Structural and functional changes in key regions such as the amygdala, hippocampus, nucleus accumbens and vmPFC suggest that this system undergoes substantial plasticity over the course of the disease. These alterations are likely to contribute to changes in pain perception, learning and coping mechanisms, potentially reinforcing the persistence of chronic OA pain.

Integration and cognitive appraisal of pain in osteoarthritis

Other important areas of the human brain cortex that are highly involved in pain perception are the insula and ACC. Both the insula and the cingulate cortex have major efferents and afferents to regions within the affective–motivational, the descending modulatory and sensory–discriminative systems, and are, thus, well-positioned to integrate sensory and affective aspects to generate a unified percept of pain. Unsurprisingly, both the insula and the ACC have been shown to be impaired in OA.

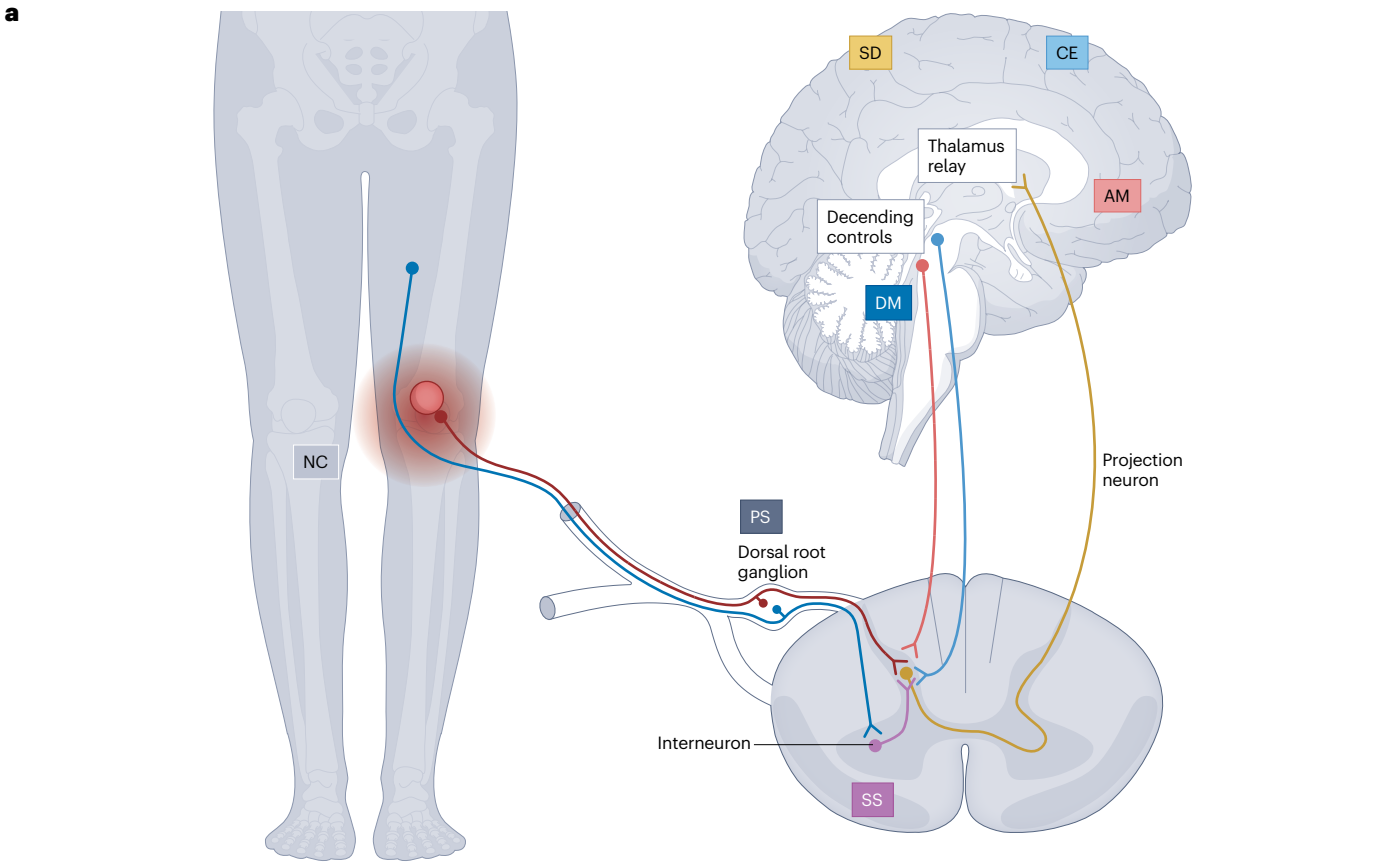
There is evidence that the volume of the insula is smaller in patients with OA than in healthy individuals[98,107,130,143], and this smaller volume has been associated with clinical composite measures of allostatic load[144], suggesting that these plastic changes are related to the continued stress and ongoing burden of the disease. Compared with the insula of healthy individuals, the insula of patients with OA also shows lower basal cerebral blood flow[101], yet greater spontaneous firing[107]. In knee OA, the anterior insula shows a loss of 'hubness' and 'degree'[93]. 'Hubness' describes the role of a region as a central hub, or a key connector, that facilitates communication between different brain regions, and 'degree' refers to the number of direct connections a region has with other areas. A loss in both 'hubness' and 'degree' means that the insula is less connected and has a compromised role in overall brain communication, which is consistent with other findings in OA showing disruptions in the functional connectivity between the insula and the medial thalamus[145], the prefrontal cortex[119] and several other cognitive and affective brain regions[138]. This integrational role is further substantiated by observations that insula activity to experimental pain is modulated by augmenting analgesic expectations[120] and when participants actively implement cognitive coping strategies[121]. The clinical implications of this altered network topography is not yet clear, but it is likely to represent a shift in how incoming nociceptive information is integrated with other affective and cognitive systems.

The ACC has been implicated in both the affective and cognitive components of pain. Patients with OA have lower ACC volumes than healthy individuals[98,130,146] and lower basal cerebral blood flow in the ACC[101]. Basal cerebral blood flow in the ACC has been correlated further with the intensity of OA pain[138]. However, pain intensity does not correlate with cingulate-cortex activity when anxiety levels are accounted for, suggesting that the modulatory role of the ACC maps into an affective and cognitive domain, rather than being purely sensory–discriminative. Converging evidence also shows that joint pain, but not experimental thermal stimuli, increases activity in the ACC of patients with OA, supporting the view that the patient's own ongoing OA pain is integrated differently by the brain[136]. Further evidence of an affective–cognitive role of the cingulate cortex comes from studies in which patients were trained to cope with their pain[121], whereby active coping led to pain reduction and proportional decreases in activity of cingulate-cortex activity. As the ACC can regulate ascending nociceptive inputs[147,148], it is also likely to be involved in top–down modulation of pain perception. It is important to note that the insula and the ACC have also been implicated in salience detection of non-painful stimuli, and so their specificity to pain has been challenged[149], potentially emerging instead as brain areas that allocate attentional resources to incoming sensory signals, including pain. Nevertheless, the allocation of attentional resources to filter relevant from irrelevant sensory inputs is likely to have a crucial role in transforming subconscious nociceptive signals into pain perception[150,151].

Taken together, the insula and ACC are key structures that integrate sensory and affective aspects of pain. The structural and functional changes observed in OA, along with involvement of these regions in pain coping and affect, emphasizes the complex interplay of cognitive and emotional factors in chronic pain.

Brain plasticity and total joint-replacement outcomes

The plastic changes in brain circuits that are associated with chronic OA pain can be further illuminated by studies comparing patients before and after TJR. Results are mixed and largely variable, probably owing to methodological variations, small sample sizes, and variable timescales for the assessment of pain outcomes. In a study comparing 32 patients with hip OA and healthy individuals, OA-associated decreases in grey matter in the amygdala, ACC, insula, prefrontal cortex and brainstem were observed; importantly, in a subset of 10 patients with OA who underwent follow-up scans 4 months after TJR, these structural abnormalities were reversed, suggesting potential plasticity in the brain structure following pain relief[130]. Two additional studies provide further insights into brain structural changes in OA patients before and after TJR. A study of 16 patients with hip OA found reduced grey matter density in the amygdala, orbitofrontal cortex, insula, ACC and thalamus before TJR compared with healthy controls. Notably, 9 months post-surgery, only the thalamus showed normalization in grey-matter density[152]. Another study involving 29 patients with knee OA revealed lower grey matter density in amygdala, primary sensory and nucleus accumbens prior to TJR than in healthy individuals[106]. Six months post-surgery, grey-matter density increased in the amygdala and hippocampus, but did not change in the nucleus accumbens, and was even further reduced in the primary sensory cortex[106]. Finally, a



magnetic resonance spectroscopy study has shown a thalamic normalization of N-acetyl aspartate, commonly interpreted as an in vivo marker of neuronal integrity, 4 weeks after total knee replacement[100].

If these brain properties reflect some level of supraspinal amplification of incoming noxious stimuli, they are also likely to hold predictive potential over the outcomes of TJR. Although the literature is still scarce, two studies have associated pre-surgical brain properties with post-surgical pain outcomes; one study featuring 81 patients with knee OA showed that the structural properties of the hippocampus, amygdala and thalamus before surgery can predict pain post-surgical outcomes 6 months after TJR[153], and the other study, which included 19 patients with knee OA, reported that increased RVM activity in response to experimental pain prior to TJR predicts the persistence of chronic post-surgical pain 12 months after TJR[117]. As both studies measured brain properties before surgery, their findings do not reflect the consequences of the procedure. Instead, they highlight

Fig. 3 | Mechanistic modelling of pain in osteoarthritis. a, We propose a mechanistic model to conceptualize pain in osteoarthritis (OA) as the aggregate of peripheral joint, peripheral and central neural components, and a modulatory component. OA pain can be represented as a weighted sum of various sensory, affective and evaluative circuits. AM, affective–motivational; CE, cognitive–evaluative; DM, descending modulation; NC, nociceptive component; PS, peripheral sensitization; SD, sensory–discriminative component; SS, spinal sensitization; **ε**, margin of error or uncertainty. **b**, Individual subject values characterize the contribution of distinct components to each person's perception of pain. By identifying the relative weights of these contributing circuits, targeted strategies and their combinations can be developed to treat OA pain more effectively. **c**, This mechanistic model of pain could help to identify subtypes of patients and facilitate the clinical management of OA pain through tailored strategies. For example, we anticipate that Patient A, who has a predominant NC of pain, might experience substantial pain relief following NSAID medications, targeted joint interventions or joint replacement surgery. By contrast, Patient B, who primarily exhibits PS or SS, might benefit from neuromodulation strategies. Patient C, with prominent AM and CE components, is likely to respond to psychotherapeutic interventions and centrally acting medications. In practice, patients are likely to exhibit abnormalities in all components of OA pain to varying degrees. In Patient D and in most cases, optimal treatment might involve a combination of strategies.

brain properties as potential risk factors for chronic OA pain. These properties might reflect a chronic state of nociceptive facilitation or alternatively reflect the impact on cognitions and behaviours, including anxiety and fear-related behaviour, leading to worse pain outcomes. Naturally, the interactions and associations between brain parameters and treatment outcomes are complex, and the exact causal role of the brain in TJR outcomes remains to be clarified.

Mechanistic modelling of osteoarthritis pain

The opportunity to investigate the distinct components that influence pain perception in OA, and their temporal changes, through the use of both animal models and translational human imaging studies, enables the identification of multiple physiological mechanisms that collectively contribute to the overall pain experience: from the molecular changes within the joint to the adaptations in the peripheral nervous system, spinal-cord dynamics, descending modulatory control and supraspinal mechanisms (Fig. 3a). Although detailed research in each of these areas is essential, pursuing a broad perspective and considering OA pain as the cumulative result of the interplay among these components is equally important.

We propose that a framework to better understand OA pain, account for individual differences and ultimately improve treatment approaches in OA would involve modelling the pathophysiological components of OA pain (Fig. 3b). This approach would enable testing of the influence of various factors on pain perception and explore individual differences by assuming that these components vary among OA patients. Consequently, this variability might be linked to distinct clinical presentations and responses to treatments. Understanding these differences would potentially clarify why standardized treatments are effective for some patient cohorts, whereas others — such as those with persistent pain following joint replacement — do not experience relief. The presented model (Fig. 3c) is a simple additive model. One could formulate a more anatomy–physiology-derived directed acyclic graph with various components interacting as moderators and/or mediators. Yet such work would need to be built on this initial model. Such models provide the opportunity to link physiology and psychology and derive surrogate clinical models with far easier applicability in the clinic.

An additional key scientific focus is on developing effective methods to measure these pain components. Various tools, applicable in both human and animal research, have the potential to illuminate specific mechanisms (Table 1), yet substantial efforts should be directed towards improving our ability to quantify each component. Another valuable method involves identifying individuals who respond well to specific treatments, then tracing back their clinical and quantitative measurements to uncover predictive markers. Together, these strategies could refine our understanding of pain mechanisms and guide more personalized treatment approaches.

Opportunities for osteoarthritis pain treatments

By constructing multi-variable models of the peripheral and central circuits involved in OA pain, we can start to uncover underlying mechanisms, identify therapeutic targets and develop probabilistic models for predicting treatment outcomes. This comprehensive approach may even enable personalized decision making in managing chronic OA pain (Fig. 3c), in which treatment decisions can be grounded in understanding each patient's unique pain pathophysiology. For instance, a patient with a predominant nociceptive component might achieve substantial pain relief from interventions targeting the peripheral source of pain, such as NSAIDs, intra-articular injections or even TJR. Conversely,

Table 1 | Monitoring specific components of the pain experience in osteoarthritis

Pain component	Monitoring tools
Nociception	Pressure or heat pain thresholds fMRI
Peripheral sensitization	Pressure or heat pain thresholds Electrodiagnosis (NCS)
Spinal sensitization	DMA Temporal summation
Sensory–discriminative circuit	Electrodiagnosis (EMG/NCS/EEG) Physical performance tests fMRI
Descending modulation	Conditioned pain thresholds Offset analgesia fMRI
Cognitive–evaluative component	Neuropsychological assessments EEG fMRI
Affective–motivational component	Psychological assessments EEG fMRI Patient-reported outcome measures

This table is intended as an example rather than an exhaustive list, as additional and diverse tools will likely enrich the scientific understanding of osteoarthritis pain. Employing a combination of strategies and integrating animal and human research in a translational and reverse-translational approach, and back-tracing characteristics of responders to various treatment methods are all strategies that can enhance our understanding of pain in osteoarthritis. DMA, dynamic mechanical allodynia; EEG, electroencephalogram; EMG, electromyography; fMRI, functional magnetic resonance imaging; NCS, nerve conduction study.

Glossary

Amygdala
A brain structure located in the medial temporal lobe, crucial for processing emotions such as fear, anxiety and pain. The amygdala has a key role in threat assessment, emotional memory formation and behaviours associated with reward and reinforcement, connecting with various regions to influence sensory responses, including nociception.

Anterior insula
A region of the insular cortex involved in processing higher-level sensory, affective and cognitive states. The anterior insula is implicated in integrating emotional responses, including those related to pain.

Central sensitization
Central sensitization is a phenomenon characterized by increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input.

Cingulate cortex
Located in the medial region of the cerebral cortex, wrapping around the corpus callosum, the cingulate cortex is involved in pain perception, emotion regulation, decision making and attention. The cingulate cortex integrates emotional, sensory and cognitive information and is divided into anterior and posterior sections with distinct roles in emotional processing and sensory integration.

Corticolimbic system
A neural network that expands upon the mesolimbic system to include cortical projections of the nucleus accumbens, incorporating regions such as the ventromedial prefrontal cortex, hippocampus and amygdala. The corticolimbic system has a role in emotional regulation and reward processing.

Dorsal horn
The sensory processing area of the spinal cord that receives input from peripheral nerves on pain, pressure and other stimuli, and transmits these signals to the brain.

Dorsolateral prefrontal cortex
A region of the prefrontal cortex involved in executive functions such as working memory, decision making and cognitive flexibility, often implicated in the cognitive modulation of pain.

Grey matter density
The concentration of neuronal cell bodies, dendrites and glial cells in a specific brain region. Higher density indicates greater numbers of neurons or synapses, often associated with neuroplasticity in response to experiences or injuries.

Hippocampus
A structure within the limbic system involved in memory formation and spatial navigation. The hippocampus interacts with regions involved in emotional processing, including the amygdala, and has a role in contextual memory associated with pain experiences.

Hypothalamus
Located near the pituitary gland, the hypothalamus regulates essential physiological processes such as body temperature, hunger, sleep and circadian rhythms. The hypothalamus coordinates stress responses and links the nervous system to the endocrine system through its control over the pituitary gland.

Mesolimbic system
A neural pathway implicated in reward, salience and conditioned learning, comprising key structures of the dopamine system, including the ventral tegmental area and the nucleus accumbens.

Motor homunculus
A somatotopic representation of the body in the brain's primary motor cortex, where different body parts are controlled by specific regions along the cortex's dorsal–ventral axis.

Negative affect
A broad psychological construct encompassing emotional distress and unpleasant feelings. It encapsulates affective dimensions such as depression, anxiety, fear and anger, frequently co-occurring with acute and chronic pain.

Nociceptors
Specialized peripheral neurons that detect noxious (painful) stimuli and transmit this information to higher-order structures in the central nervous system.

Nucleus accumbens
Part of the brain's reward circuitry within the basal ganglia, involved in processing reward, motivation and pleasure. The nucleus accumbens has a role in reinforcing behaviours associated with pain relief.

Periaqueductal grey area
(PAG). A midbrain region involved in pain modulation, defensive behaviours and autonomic functions. The PAG integrates sensory information related to pain and, through connections with the rostroventral medulla, can inhibit pain signals in the spinal cord.

Posterior insula
A part of the insular cortex located within the brain's lateral sulcus. The posterior insula processes sensory information related to the body's internal state, such as nociception, temperature and visceral sensations, contributing to physical sensation perception.

Primary and secondary sensory cortices
Located in the parietal cortex, this region is essential for processing sensory information from the body, including touch, pressure, pain and proprioception. Sensory cortices receive input from the thalamus.

Primary motor cortex
The primary motor cortex is a region of the cerebral cortex located in the frontal lobe, specifically on the precentral gyrus and anterior paracentral lobule. It is the main area responsible for initiating and controlling voluntary movements.

Quantitative sensory testing
A standardized psychophysical method used to assess somatosensory function. Quantitative sensory testing helps to characterize both peripheral and central nervous system function through the application of calibrated stimuli and subsequent evaluation of various sensory modalities, including thermal, mechanical sensation and pain, and vibration sensation.

Rostroventral medulla
(RVM). A brainstem area involved in descending pain modulation, controlling the transmission of pain signals between the brain and spinal cord. The RVM can either inhibit or enhance pain signals through its connections with the dorsal horn.

Spinal sensitization
A process involving structural and functional changes in the spinal cord that amplify pain signals. Prolonged nociceptor activation leads to increased excitatory signalling and reduced inhibition, causing heightened pain sensitivity and persistent pain perception.

Glossary (continued)

Spinothalamic pathway
A major ascending pathway that originates in the dorsal horn of the spinal cord and projects to the thalamus, carrying sensory information, including pain, to the brain.

Spontaneous neural activity
Ongoing, intrinsic fluctuations in neuronal firing that occur independently of explicit tasks or stimuli, representing baseline brain function. In functional magnetic resonance imaging, these are predominantly low-frequency (<0.1 Hz) fluctuations in the blood oxygen level-dependent signal captured with the MRI protocol, reflecting underlying neural processes and their associated metabolic demands.

Thalamus
A brain structure that acts as a relay centre for incoming sensory information, including pain. The lateral thalamus primarily projects to sensory brain areas, whereas the medial thalamus projects to regions involved in emotional and cognitive responses to pain.

Ventromedial prefrontal cortex
(vmPFC). A region in the lower middle part of the prefrontal cortex that is involved in decision making and emotional regulation by integrating emotional and value-based information, especially related to risk, reward and guiding behaviour.

a patient who displays a prevailing component of peripheral sensitization or spinal sensitization might benefit from neuromodulation strategies tailored to modulate these sensitization processes, such as transcutaneous and peripheral nerve stimulation, or dorsal root ganglion stimulation. Similarly, patients whose pain is characterized by prominent affective–motivational and cognitive–evaluative components might respond more effectively than others to psychotherapeutic interventions, such as cognitive behavioural therapy, centrally acting medications that address the emotional and cognitive aspects of pain, such as duloxetine and other antidepressive drugs, and central neuromodulation approaches, including direct cranial stimulation and transcranial magnetic stimulation. Although neuromodulation approaches show promise in treating OA pain, with emerging evidence supporting their potential efficacy, there is still a lack of data regarding its application. Peripheral nerve stimulation has demonstrated analgesic effects in patients with knee OA[154,155] and dorsal root ganglion stimulation has demonstrated analgesic effects in animal models of OA[156] and in patients with knee OA and intractable joint pain[154,155,157]. In addition, transcranial magnetic stimulation is currently being studied as a possible therapeutic option[158,159]. Large, well-designed randomized controlled trials are necessary to robustly establish the efficacy, safety and long-term benefits of these neuromodulation techniques for OA pain management.

In practice, patients might often exhibit varying degrees of multiple components, necessitating a combination of treatment strategies. The challenge lies in identifying the relative contributions of each component and mapping the relevant parameters to measure them accurately. Nonetheless, this mechanistic approach holds potential for improving OA pain management by creating tailored treatment strategies that align with each patient's specific pain profile. An important concept to consider is that chronic pain, such as pain associated with OA, is intrinsically linked to neuroplasticity. Consequently, pain mechanisms evolve over time, as previously discussed in this Review. Therefore, mechanistic models of pain might also change over time, and central and peripheral mechanisms might have distinct contributions to pain at distinct stages of OA pain.

Limitations of the proposed model stem from the challenges in obtaining objective measurements for each term in the equation. Additionally, translating the scientific metrics used to define these terms into practical applications is complex. For a mechanistic model of OA pain to have clinical utility, these metrics would need to be made accessible for routine use in clinical settings, which implies further effort in identifying potential surrogate markers that can be applicable on a large scale with lower costs for stakeholders. Developing such clinically applicable metrics will require bridging the gap between theoretical constructs and measurable clinical biomarkers, ensuring that they are both feasible for clinicians to implement and reliable in reflecting the model's underlying concepts.

Conclusions

Here, we reviewed the evidence that joint pain is highly prevalent and does not entirely reflect the extent of joint injury in OA. Consistent with this viewpoint, current advances highlight the expanded role of brain affective–motivational, cognitive–evaluative and descending modulation circuits in chronic joint pain. Various brain regions exhibit adaptive or maladaptive changes in response to chronic joint pain, which we propose can be summarized in a multidimensional model. Such models would substantially advance the field by providing quantitative biomarkers to predict the properties and future course of joint pain, both with and without treatment, at a group level and an individual patient level. Although much remains to be done, numerous studies have firmly established that joint pain cannot be regarded as a straightforward reflection of joint injury. Instead, the evidence indicates that the characteristics of joint pain are consistent with and complementary to those of other chronic pain conditions.

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Review article

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IgG4-related disease and other fibro-inflammatory conditions

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Abstract

IgG4-related disease (IgG4-RD) is a fibro-inflammatory disorder usually characterized by multi-organ involvement. Its pathogenesis is complex and involves genetic and environmental factors, while immune responses usually mediate organ damage and promote fibrosis, which is a key feature of the disease. IgG4 responses, however, are not exclusive to IgG4-RD and can be encountered in other diseases with phenotypes that partially overlap that of IgG4-RD. Although IgG4-RD has clinical and histological hallmarks, the lack of validated diagnostic criteria often makes the diagnosis challenging, requiring a multi-dimensional approach that integrates clinical, radiological and serological data. The present Review covers recent advances in the understanding of disease drivers and its clinical phenotypes, mainly focusing on the differential diagnosis with potential IgG4-RD mimickers, namely histiocytoses, lymphoproliferative disorders, systemic vasculitides and other immune-mediated conditions. The Review also provides a schematic approach to IgG4-RD treatment, including a brief overview of glucocorticoid-sparing agents and emerging therapies, from B cell-depleting monoclonal antibodies to cytokine-targeting drugs, the majority of which are currently under investigation in randomized clinical trials.

Sections

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Key points

- IgG4-related disease (IgG4-RD) is a fibro-inflammatory disease characterized by slow-growing and often pseudotumoural lesions that can be solitary or occur in multiple organs.
- The diagnosis of IgG4-RD requires the exclusion of a wide array of neoplastic, infectious and autoimmune disorders as well as of rare proliferative conditions such as histiocytoses and Castleman disease.
- The different subphenotypes of IgG4-RD (Mikulicz, head-and-neck limited, pancreato-hepato-biliary, retroperitoneal and/or aortic disease) differ in terms of patients' demographic features, clinical manifestations and serum IgG4 levels.
- Treatment of IgG4-RD is based on the use of glucocorticoids, but B cell-depleting therapies (for example, rituximab or inebilizumab) are being incorporated into the standard therapeutic regimens.
- IgG4-RD is a chronic–relapsing disorder and therefore requires careful and long-term follow-up.

Introduction

The initial report of an 'IgG4-associated' disease dates back to 2001, when Hamano et al.[1] showed that patients with autoimmune pancreatitis had polyclonal hypergammaglobulinaemia with a marked increase in IgG4. In 2002, the same group demonstrated $IgG4^+$ plasma-cell infiltration of the pancreatic and ureteral lesions of individuals with autoimmune pancreatitis associated with retroperitoneal fibrosis[2]. In 2003, Kamisawa et al.[3] reported that, in patients with sclerosing pancreatitis, $IgG4^+$ plasma-cell infiltration could also affect other sites of the digestive tract (for example, the biliary tree, stomach or colon) as well as remote organs such as the salivary glands, lymph nodes and bone marrow, thus introducing the concept of a systemic disease. It then became clear that several seemingly distinct entities, including sclerosing pancreato-cholangitis, Riedel thyroiditis, Mikulicz disease (chronic dacryoadenitis and sialoadenitis) and orbital pseudotumour could be grouped under the umbrella of what was defined as 'IgG4-related disease' (IgG4-RD). These entities all shared key pathological features, namely pseudotumoural lesions, $IgG4^+$ plasma-cell infiltration, fibrosis and chronic lymphoplasmacytic inflammation[4].

During the past two decades, considerable advances were made in understanding the pathophysiology and clinical aspects of IgG4-RD. However, owing to the lack of validated diagnostic criteria and disease-specific biomarkers, the boundaries of the IgG4-RD spectrum are not yet well defined and several diagnostic issues have arisen. Fibro-inflammatory lesions that often lack a prominent IgG4 response (that is, tissue infiltration by $IgG4^+$ plasma cells and/or increased serum levels of IgG4), such as pachymeningitis and retroperitoneal fibrosis, are nevertheless considered to be IgG4 related because of their histological and clinical similarities to typical IgG4-RD lesions[5]; conversely, pronounced IgG4 responses have been recognized in other inflammatory conditions (for example, idiopathic multicentric Castleman disease (iMCD)), whose clinical manifestations can mimic those of IgG4-RD[6]. Finally, substantial overlap has emerged between IgG4-RD and other autoimmune or proliferative disorders such as histiocytoses and systemic vasculitis[7,8]. These and other aspects make IgG4-RD a puzzling condition and have implications for its diagnosis and management. Herein, we review the latest developments in IgG4-RD clinical phenotyping, pathophysiology and management, with a focus on the main disease mimickers and on how to approach the challenging issues related to differential diagnosis.

Clinical presentation and disease phenotypes

General features of IgG4-RD

IgG4-RD is a systemic, immune-mediated, fibro-inflammatory disorder. It usually has an insidious onset and can lead to silent and irreversible organ damage. The clinical presentation of IgG4-RD differs from that of other systemic autoimmune conditions with a rapidly progressive course such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis or giant-cell arteritis (GCA). However, other conditions that mimic IgG4-RD, such as histiocytosis, can also have an indolent presentation. IgG4-RD occurs more frequently in men than in women (male:female ratio 2:1–3:1), with men usually experiencing more severe disease[9].

IgG4-RD has clinical and histological hallmarks. The lesions usually have a pseudo-tumoural growth pattern and often present as tumour-like masses; organ damage can result from the compressive effects that these lesions exert on neighbouring structures (for example, ureteral obstruction by retroperitoneal fibrosis or common bile-duct obstruction by focal sclerosing pancreatitis). In other instances, organ damage is attributable to diffuse parenchymal infiltration, as occurs in IgG4-related tubulointerstitial nephritis or diffuse sclerosing pancreatitis[10]. Another distinguishing feature of IgG4-RD is its vascular tropism: involvement of large and medium-sized arteries such as the aorta and the carotid, mesenteric and coronary arteries is seen in approximately 50% of cases (Table 1). Interestingly, small vessels can also be involved: fibrous sheathing of arterioles or capillaries and inflammation of small veins are also frequent[11]. These findings suggest that IgG4-RD can be a vasculitis affecting vessels of variable size[12–14]. Histologically, IgG4-RD lesions almost invariably harbour fibrous and chronic inflammatory components (Fig. 1), with their relative proportions accounting for the different activity of the lesions on metabolic imaging studies (such as ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET–CT[15]. The coexistence of fibrosis and chronic inflammation is almost a prerequisite for the diagnosis of IgG4-RD.

Main clinical manifestations of IgG4-RD

The most common IgG4-RD lesions are listed in Table 1. A detailed analysis of clinical manifestations and radiology findings is beyond the scope of this Review. The main clinical signs and symptoms depend on which organs are affected and can be extremely variable (Fig. 2), further complicating the differential diagnosis. Pancreato-biliary involvement (Fig. 2e) can cause jaundice, pruritus, abdominal pain, steatorrhea and new-onset diabetes mellitus[16]. Bilateral swelling of lacrimal and major salivary glands indicates Mikulicz disease[17], whereas other commonly affected sites in the head and neck include cervical lymph nodes, thyroid[18], ocular tissues and annexes, extraocular muscles, orbital fat and trigeminal nerve, possibly manifesting with chronic swelling of the orbit and proptosis[19] (Fig. 2b). Skull-base involvement can also occur, with cranial neuropathies being its most common presentation[20]. Both thoracic and abdominal peri-aortitis, as well as peri-arteritis of the main aortic branches (for example, epi-aortic vessels), are frequent. Abdominal peri-aortitis can range from thin peri-aortic and/or peri-iliac sheathing to large peri-aorto-iliac masses that can encase the ureters, traditionally

Table 1 | Clinical manifestations of IgG4-RD and its main mimics[7,22,24,25,84–110]

Characteristic	IgG4-RD	ECD	iMCD	Sarcoidosis	Large-vessel vasculitis	GPA
Median age at diagnosis	55–65 years	45–55 years	50–55 years	45–50 years	TA: 25–35 years; GCA: 70–80 years	50–60 years
Male-to-female ratio	1.5–4:1	2.5–3:1	0.5–1.5:1	0.5–1:1	0.1–0.25:1	1–1.5:1
Head and neck						
Pachymeningitis	Very rare	Rare	Very rare	Very rare	Very rare	Rare
Pituitary involvement	Very rare	Moderately frequent	Very rare	Very rare	Very rare	Very rare
Retro-orbital infiltration	Rare	Moderately frequent	Very rare	Very rare	Very rare	Rare
Lacrimal and/or salivary gland involvement	Frequent	No data available	Very rare	Very rare	No data available	Rare
Chronic rhinosinusitis	Rare	Frequent	No data available	Very rare	No data available	Frequent
Chest						
Thoracic (peri-)aortitis	Rare	Frequent	No data available	Very rare	Moderately frequent	Rare
Mediastinitis	Very rare	Rare	Rare	Very rare	No data available	No data available
Lung involvement	Rare	Moderately frequent	Frequent	Frequent	Very rare	Frequent
Cardiac involvement	Rare	Moderately frequent	No data available	Very rare	Very rare	Rare
Abdomen						
Hepato-biliary involvement	Rare	Very rare	Rare	Rare	Very rare	Very rare
Spleen involvement	Very rare	Very rare	Frequent	Rare	No data available	Very rare
Pancreatitis	Frequent	Very rare	Very rare	Very rare	No data available	Very rare
Mesenteritis	Very rare	Rare	No data available	Very rare	Rare	No data available
Retroperitoneal fibrosis (peri-aortic and/or peri-iliac)	Moderately frequent	Frequent	Very rare	Very rare	Rare	Very rare
Peri-renal fibrosis	Very rare	Frequent	No data available	Very rare	No data available	No data available
Parenchymal renal involvement	Rare (80% TIN, 15% MN)	No data available	Very rare (case reports of renal mass or GN)	Very rare (80% TIN)	No data available	Frequent (GN)
Other						
Skin lesions	Very rare	Moderately frequent	Rare	Rare	Very rare	Moderately frequent
Bone involvement	Very rare	Frequent	Rare	Very rare	Very rare	No data available
Lymphadenopathy	Moderately frequent	Rare	Frequent	Frequent (thoracic)	No data available	Rare

'Very rare' manifestations have been reported in case reports or have a frequency <10%; 'rare' manifestations have a frequency <25%; 'moderately frequent' manifestations have a frequency 25–50%, and 'frequent' manifestations have a frequency >50%. ECD, Erdheim–Chester disease; GCA, giant cell arteritis; GN, glomerulonephritis; GPA, granulomatosis with polyangiitis; IgG4-RD, IgG4-related disease; iMCD, idiopathic multicentric Castleman disease; MN, membranous nephropathy; TA, Takayasu arteritis; TIN, tubulointerstitial nephritis.

referred to as retroperitoneal fibrosis[13,21]. Typical symptoms of retroperitoneal fibrosis include abdominal, flank or lumbar pain, lower-limb oedema, lower urinary tract symptoms, mild fever and weight loss[21]. Retroperitoneal fibrosis can also develop at atypical sites such as the pre-sacral area or the peri-renal space (Fig. 2). The renal parenchyma can be affected by IgG4-related tubulointerstitial nephritis, which is usually characterized by mild proteinuria and variable degrees of kidney-function impairment[22,23]. Less frequent manifestations include non-specific skin lesions, mesenteritis (Fig. 2h), pachymeningitis, hypophysitis, lung involvement and prostatitis[10].

IgG4-RD sub-phenotypes

IgG4-RD is clinically heterogeneous and comprises different sub-phenotypes. A cluster analysis run on an international cohort including 493 patients identified four distinct disease sub-phenotypes: pancreato-hepato-biliary disease, retroperitoneal fibrosis and/or aortitis, disease limited to the head and neck and classic Mikulicz disease with systemic involvement[24]. The different groups were primarily defined by the different distribution of organ involvement. Nevertheless, other notable differences were highlighted: for instance, the head-and-neck-limited disease cluster was characterized by a predominance of

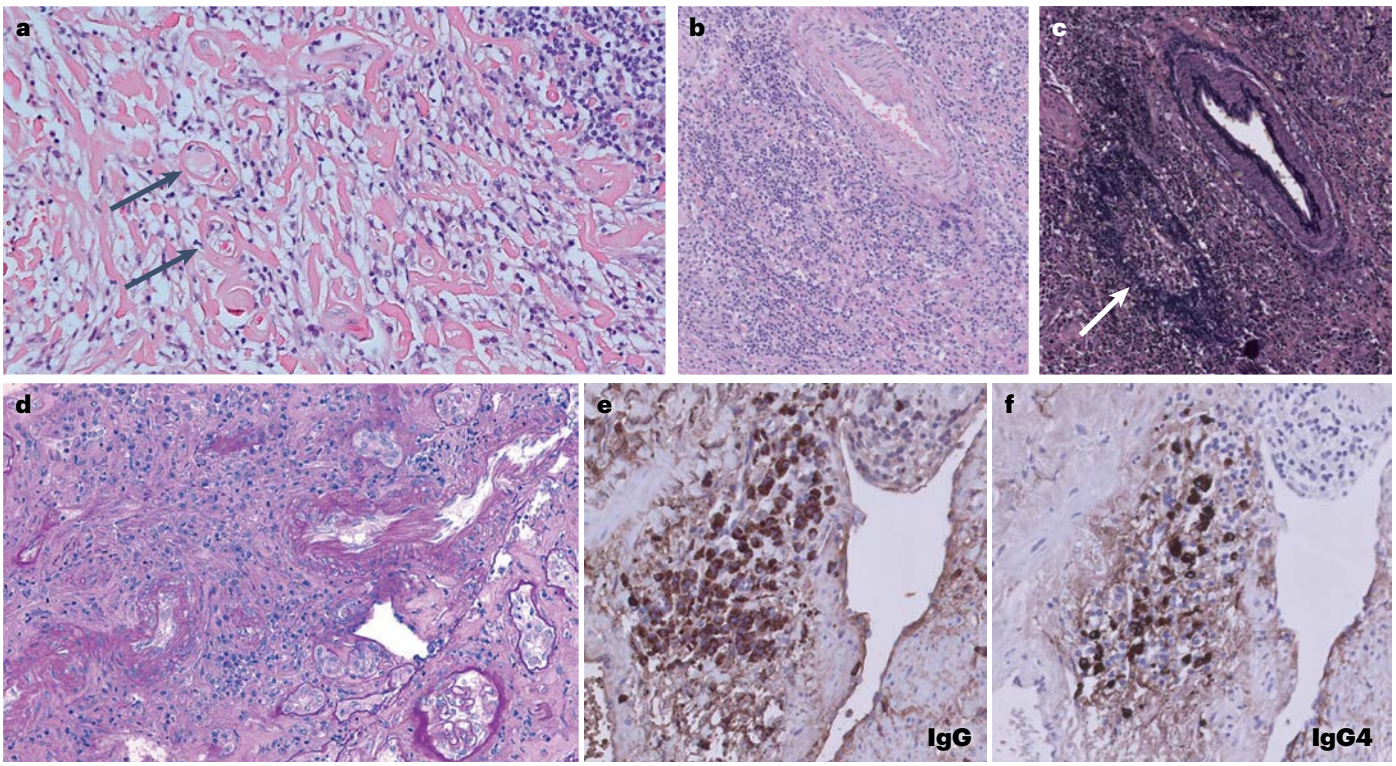

Fig. 1 | Main histological findings in IgG4-related disease. a, Retroperitoneal tissue from a patient with retroperitoneal fibrosis shows pronounced fibrosis with irregular distribution of the fibrous bundles, which often encircle small vessels (arrows) (haematoxylin and eosin (H&E) stained; original magnification ×20). **b**, Retroperitoneal tissue showing a dense lymphoplasmacytic infiltrate and an intact artery (stained with H&E; original magnification ×10). **c**, The same tissue area shown in **b**, in a section stained with Elastica van Gieson, reveals the presence of obliterative phlebitis (arrow) (original magnification ×10). **d**, Kidney tissue from an individual with IgG4-related tubulointerstitial nephritis showing interstitial storiform fibrosis and a chronic tubulointerstitial inflammatory infiltrate (periodic acid-Schiff stained; original magnification ×20). **e**, IgG staining in salivary-gland tissue of a patient with IgG4-related sialoadenitis showing IgG-positive cells (anti-IgG antibody stained; original magnification ×20). **f**, The same biopsy area shown in **e** reveals that a substantial proportion of cells are IgG4-positive (anti-IgG4 antibody stained; original magnification ×20).

younger, female and Asian individuals, whereas white male patients more frequently had hepato-biliary involvement and/or peri-aortitis. The highest serum IgG4 levels were found in the Mikulicz's disease with systemic involvement cluster, the lowest in the retroperitoneal fibrosis cluster[24] (Supplementary Table 1). Other studies on IgG4-RD sub-phenotypes found similar results, with multisystemic disease being characterized by higher serum IgG4 levels[5,25].

Within the past year, a distinction between fibrotic and proliferative (or inflammatory) features of IgG4-RD has been proposed[26,27]. Retroperitoneal fibrosis, mediastinitis, Riedel's thyroiditis, orbital pseudotumour and pachymeningitis were classified as fibrotic manifestations, whereas pancreato-biliary, lacrimal-salivary and kidney involvement were included among the proliferative manifestations. This separation is based on several assumptions: the elevation of serum levels of IgG, IgG4 and inflammatory markers is less prominent in the fibrotic subtype than in the proliferative subtype; fibrotic lesions have only scanty inflammatory infiltrates and low-to-absent metabolic activity on PET; and the improvement of fibrotic lesions (in terms of size and metabolic activity) in response to glucocorticoids or B cell targeting therapies is less brilliant than that seen with proliferative lesions. Although these considerations might hold true for specific manifestations (for example, pachymeningitis), other manifestations, such as retroperitoneal fibrosis, mediastinitis and orbital involvement, are usually metabolically active and respond swiftly to therapy. Thus, we believe that such a distinction should not be clearcut and, more importantly, should not discourage the treatment of fibrotic lesions.

Pathogenesis

IgG4-RD is a complex disorder. Genetic, environmental and lifestyle-related factors confer predisposition to the disease, and its immunopathogenesis involves complex immune responses orchestrated by T cells, B cells and plasma cells (Fig. 3). Varying combinations of genetic and environmental agents, together with different potential autoantigens, could account for the clinical diversity of IgG4-RD, although no studies have compared the pathogenic drivers of the different disease sub-phenotypes[23].

Genetic susceptibility and other risk factors

The genetic susceptibility to IgG4-RD has not been extensively investigated, probably owing to the rarity of the disease and the paucity of familial cases. Common variants of individual susceptibility genes such as *CTLA4*, *PRSS1*, *SPINK1* and *FGFBP2* have been identified in

small cohorts, but most of these studies included mainly patients with predominant pancreatic involvement[28–32]. In a 2024 report of familial IgG4-RD, all three affected family members shared variants of the transcription factor IKAROS, encoded by *IKZF1*, and of the E3 ubiquitin ligase UBR4, encoded by *UBR4*. Both variants were found to functionally enhance T cell activation and T helper 2 (T_H2) responses, and UBR4 was associated with IgG4 class-switch[33].

Large-scale genetic studies have also been performed. A genome-wide association study involving 850 Japanese individuals with IgG4-RD identified *HLA-DRB1* and *FCGR2B* as susceptibility loci[34]. Another genome-wide study performed using the Immunochip platform in 327 patients with retroperitoneal fibrosis clearly showed that the disease-associated locus was *HLA-DRB1*03*, a traditional marker of autoimmune diseases[35]. Notably, this study also showed that the amino acid variant Arg74, which is structurally involved in the peptide-binding groove of the HLA-DRβ molecule, was associated with disease susceptibility[35].

Regarding the association between environmental agents and IgG4-RD, exposure to industrial oils, metals and cigarette smoking is frequently reported in patients' history, although compelling evidence

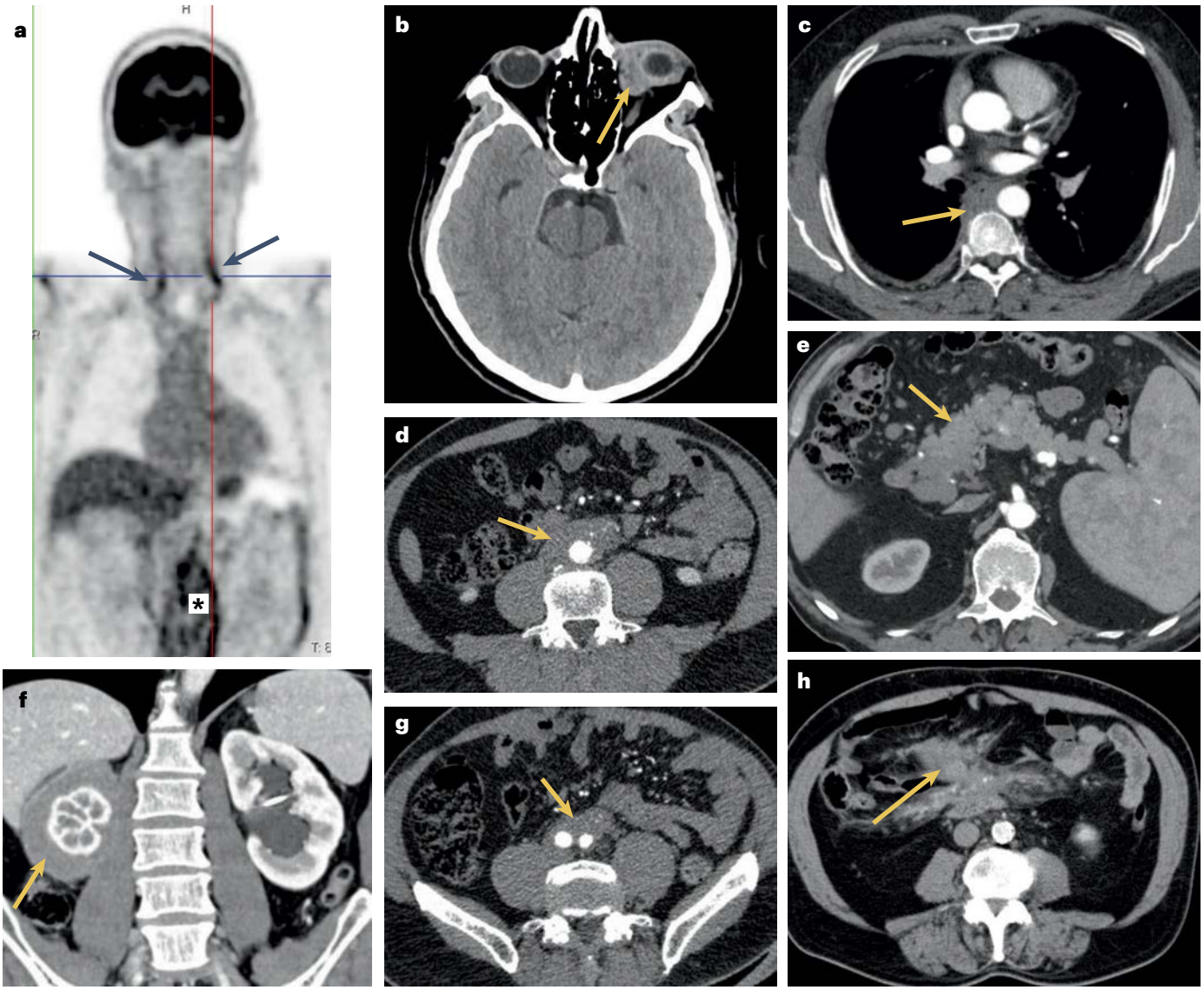

Fig. 2 | Radiological findings in IgG4-related disease. a, PET image (coronal view) showing increased uptake of fluorodeoxyglucose around the abdominal aorta (asterisk) and the common carotid arteries (arrows). **b**, Head CT image (axial view) showing orbital involvement (arrow). **c**, CT image of the chest (axial view) showing mediastinal fibrosis, which appears as a pre-vertebral, muscle-isodense tissue (arrow) adjacent to the descending thoracic aorta. **d**, Abdominal CT image (axial view) showing peri-aortitis (arrow) developing on the antero-lateral sides of the abdominal aorta. **e**, Abdominal CT image (axial view) showing diffuse enlargement of the pancreas due to sclerosing pancreatitis (arrow). **f**, Abdominal CT image (coronal view) showing a right-sided peri-renal tissue (arrow) due to IgG4-related disease, peri-renal infiltration, as well as left-sided hydronephrosis (secondary to retroperitoneal fibrosis). **g**, In the same case shown in **d**, the peri-aortic tissue also surrounds both iliac arteries (arrow). **h**, Abdominal CT image (axial view) showing diffuse thickening of the mesentery due to sclerosing mesenteritis (arrow).

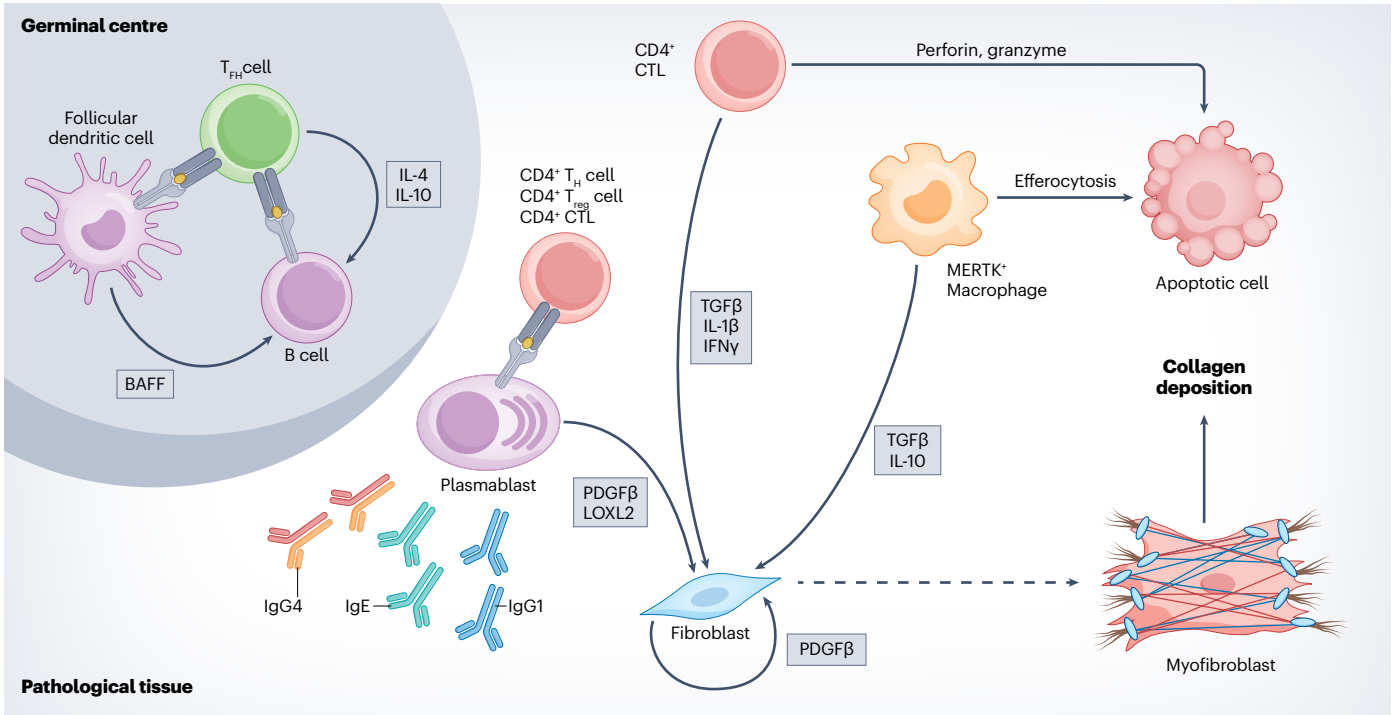

Fig. 3 | Immunopathogenesis of IgG4-related disease. Collagen deposition within pathological tissues in IgG4-related disease results from the activation of fibroblasts through a complex crosstalk involving activated B cells, plasmablasts, $CD4^+$ T cells and macrophages. Within germinal centres, oligoclonal expansion of autoreactive B cells is promoted via the presentation of autoantigens by T follicular helper (T_{FH}) cells and follicular dendritic cells. T_{FH} cells are thought to drive IgG4 class-switching through the production of IL-10 and IL-4, together with B cell activating factor (BAFF)-producing dendritic cells. Activated B cells migrate into pathological tissues, eventually promoting $CD4^+$ T lymphocyte activation and differentiation. $CD4^+$ cytotoxic T lymphocytes (CTLs) determine apoptosis within infiltrated tissues by releasing pro-apoptotic molecules (e.g. perforin and granzyme); moreover, they contribute to the activation of resident fibroblasts through the expression of transforming growth factor-β (TGFβ), IL-1β and IFNγ. $MERTK^+$ macrophages clear apoptotic cell debris through efferocytosis and release IL-10 and pro-fibrotic molecules such as TGFβ. Oligoclonally expanded B cells and plasmablasts also contribute to fibroblast activation through the secretion of platelet-derived growth factor-β (PDGFβ) and lysyl oxidase homologue 2 (LOXL2). Plasmablasts and plasma cells secrete increased amounts of IgG1 and IgG4 in the majority of patients, and increased amounts of IgE in a subset of them. MERTK, proto-oncogene tyrosine-protein kinase MER; T_H cell, T helper cell; T_{reg} cell, regulatory T cell.

of causality is lacking[36,37]. By contrast, case–control studies in patients with retroperitoneal fibrosis have consistently shown that exposure to asbestos and smoking is associated with a high risk of developing the disease, and that co-exposure has a multiplicative effect on risk[38,39].

Autoantigens, autoantibodies and the role of IgG4

The increase in serum levels of IgG4 and other IgG subclasses (especially IgG1) and the oligoclonal expansion of plasmablasts and plasma cells in affected tissues have been regarded as proof of autoimmunity in IgG4-RD[40,41]. Yet, the search for autoantigens has failed to identify a unique causative candidate and rather disclosed a variety of self-antigens including carbonic anhydrase II and IV, lactoferrin, amylase-α-2A, pancreatic trypsinogens and, more recently, galectin-3, annexin-A11, laminin-511 and prohibitin[42–44]. Although this evidence suggests a broad breakdown of immunological tolerance in IgG4-RD, each of the autoantibodies against the aforementioned antigens is found in less than one-third of patients and are also found in other autoimmune disorders; thus, they lack adequate specificity for IgG4-RD[45]. It goes without saying that their pathogenic role is as yet unproven. Nonetheless, people with IgG4-RD who produce antibodies against more than one autoantigen present with more prominent IgG elevations, complement consumption and visceral-organ involvement than those who have antibodies against one or no autoantigens, suggesting that broader autoimmunity might be associated with more severe disease[45]. The pathogenicity of the IgG4 subclass remains controversial. In general, IgG4 acts as a neutralizing antibody in autoimmune, infectious or allergic conditions, being responsible for the containment of immune responses[46,47]. In contrast to other IgG subclasses, IgG4 molecules harbour an inhibitory Fc portion that does not activate complement and poorly engages activating Fcγ receptors[48]. Accordingly, in a mouse model of autoimmune pancreatitis, patient-derived IgG4 mitigated IgG1-induced pancreatic and salivary gland inflammation[49]. In contrast to myasthenia gravis and pemphigus vulgaris, a direct contribution of IgG4 to the pathology of IgG4-RD seems unlikely. The switch from more abundant IgG subclasses to IgG4 is driven by T_H2 and regulatory T cell responses[4]; however, it is still unclear why some IgG4-RD manifestations have more prominent IgG4 signatures than others.

B cells, T cells and other cell subtypes

The landscape of immune cells in IgG4-RD has been investigated in detail, with particular attention to the B cell and T cell compartments and their contribution to tissue fibrosis. The observation of clinical improvement following treatment with B cell targeting therapies

provides the most compelling evidence to implicate B cells as drivers of IgG4-RD[50–52]. Among the many B cell subsets studied, circulating plasmablasts and $IgD^{-}CD27^{-}$ double-negative B cells were found to be oligoclonally expanded in patients with IgG4-RD, to infiltrate tissues, and to secrete pro-fibrotic molecules such as platelet-derived growth factor β and lysyl oxidase homologue 2 (refs. 41,53,54). Yet, because many aspects of B cell responses depend on collaboration with T helper cells, single-cell sequencing and multicolour immunofluorescence have been used to examine T cell subsets of interest for cognate B cell activation. In this regard, four distinct T cell phenotypes have been identified in patients with IgG4-RD, including $LAG3^{+}$ and $BATF^{+}$ T follicular helper cells and $CD4^{+}$ and $CD8^{+}$ cytotoxic T lymphocytes (CTLs)[55–58]. T follicular helper cells might drive IgG4 class-switching and B cell responses in lymphoid follicles via the production of IL-10 and IL-4 in combination with BAFF-producing dendritic cells[56,58]. CTLs contribute to tissue damage and fibrosis by inducing apoptosis and by secreting pro-fibrotic molecules such as TGFβ, IL-1β and IFNγ[59]. The reduction of circulating CTLs following B cell-directed therapy does indeed support the concept that B cells act as antigen-presenting cells to these pathogenic T cells[60]. In addition to B cells and T cells, MERTK-expressing macrophages also infiltrate the affected tissues[61]. These $MERTK^{+}$ macrophages probably efferocytose apoptotic cells and produce pro-fibrotic cytokines, thus participating in the resolution of inflammation and fuelling tissue fibrosis[61] (Fig. 3).

Diagnosis

IgG4-RD is usually a systemic disease, but it can also be limited to single organs. In such cases, the diagnosis is more challenging, especially when a biopsy cannot easily be performed or when serum levels of IgG4 are normal or only slightly increased[62]. In addition, an increase in serum IgG4 level is neither sensitive nor specific for IgG4-RD[5,62], and the typical histopathological features of the disease can also be encountered in other conditions whose clinical manifestations overlap with those of IgG4-RD[6,63,64]. Therefore, the diagnostic approach requires a combination of clinical, serological and histological findings, and the exclusion of a wide array of mimics.

Classification and diagnostic criteria

The American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR) proposed in 2019 a shared classification algorithm for IgG4-RD[65]. This algorithm requires that a patient with suspected IgG4-RD shows typical (for example, tumour-like) involvement of at least one of the 11 most commonly affected organs ('entry criteria'; Fig. 4) or typical pathology at these sites. Second, a set of 32 clinical, serological, radiological and pathological 'exclusion criteria' must be checked (Fig. 4); if any exclusion criterion is met, the patient cannot be classified as having IgG4-RD. The exclusion criteria suggest alternative diagnoses, although some of them (such as splenomegaly or lack of response to glucocorticoids) have poor specificity. If a patient meets one or more entry criteria and has no exclusion criteria, then eight 'inclusion criteria' domains (comprising clinico-pathological, serological and radiological items) must be scored. A 20-point score threshold has a specificity of ~98% and a sensitivity of ~82% for IgG4-RD classification[65]. In a Chinese study that externally validated these criteria, their specificity was 98% but their sensitivity was only 77%[66]. It is important to underline that such criteria were developed only for the purpose of classification (that is, to create homogeneous patient cohorts for clinical studies) and not for diagnostic purposes. However, even when used for classification, they have limitations. First, if the disease is confined to atypical sites (for example, the musculoskeletal system or sino-nasal structures)[67–69], it cannot be classified as IgG4-RD because it would not meet the entry

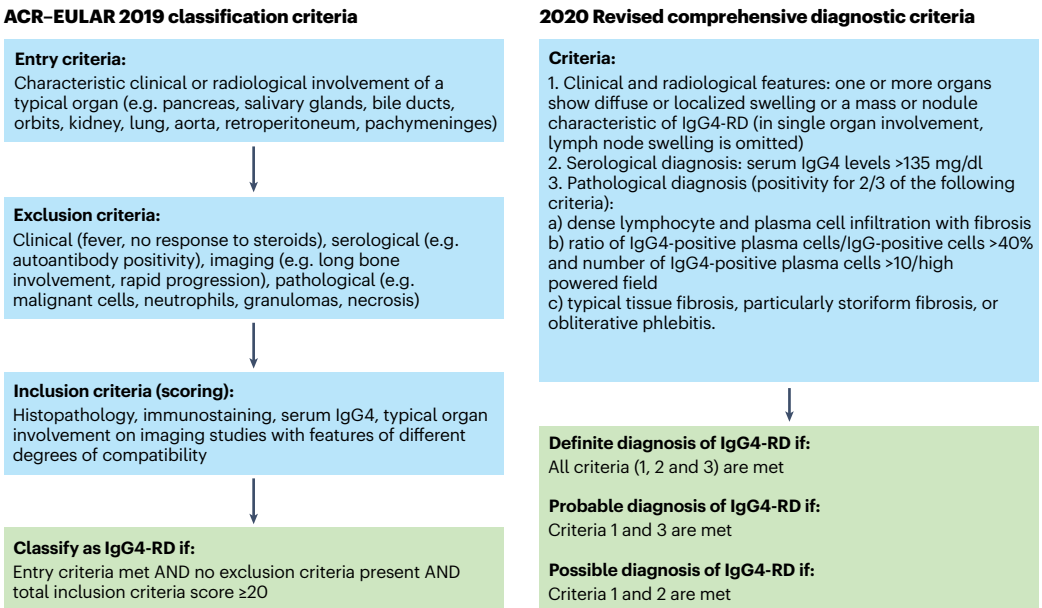

Fig. 4 | Classification and diagnostic criteria for IgG4-RD. The classification of IgG4-related disease (IgG4-RD) according to 2019 American College of Rheumatology–European League Against Rheumatism (ACR–EULAR) criteria[65] follows a three-step process, with successive entry criteria, exclusion criteria and scored inclusion criteria. The patient is classified as having IgG4-RD if a total score ≥20 is reached. The application of these criteria enables the categorization of patients for research studies. The revised comprehensive diagnostic criteria for IgG4-RD[76], published in 2020, comprise three different domains; a diagnosis of IgG4-RD is considered definite when patients fulfil the criteria in all three domains.

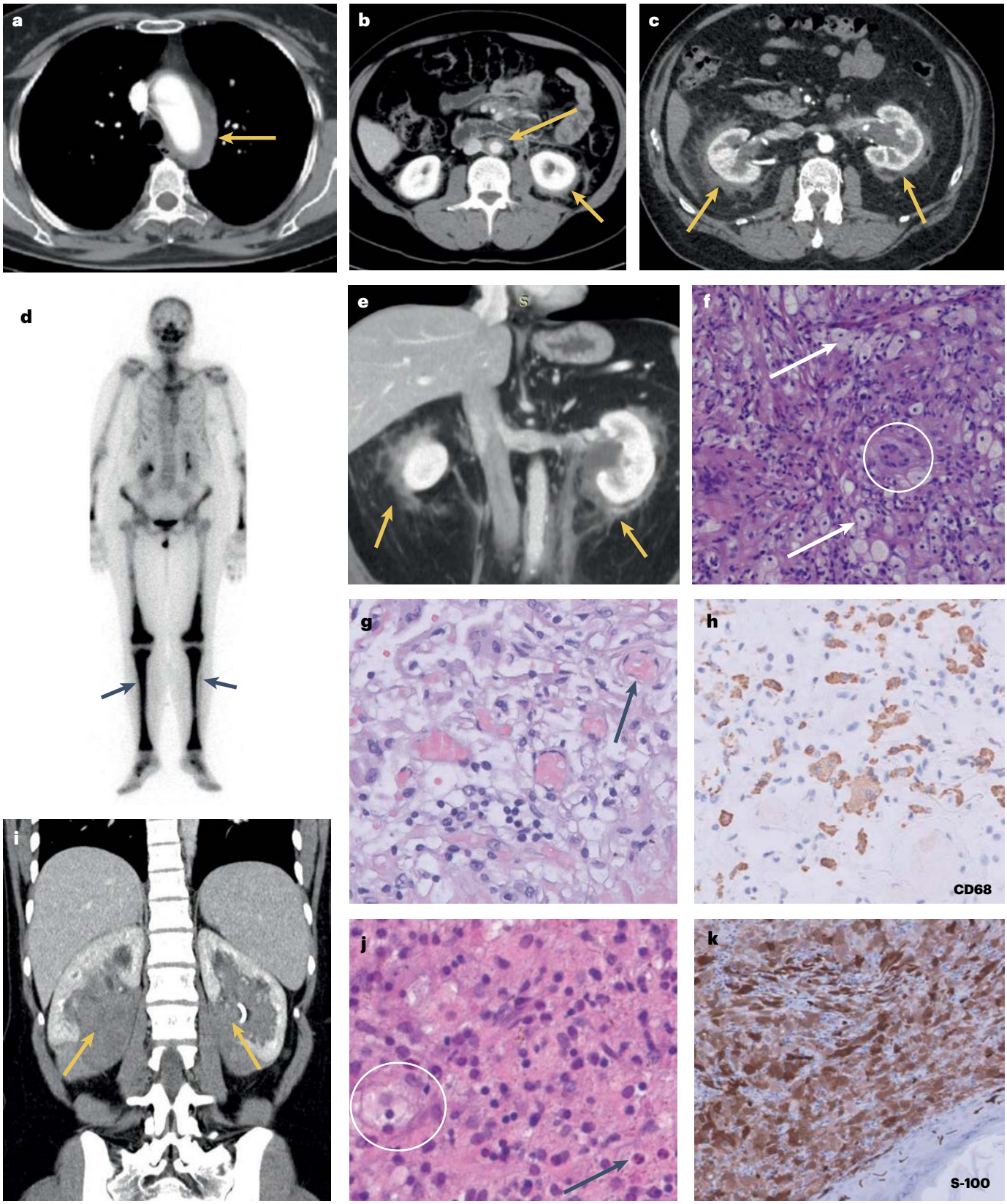
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Fig. 5 | Radiological and histopathological findings in histiocytoses mimicking IgG4-related disease. a, Chest CT scan (axial view) showing thoracic peri-aortitis involving the aortic arch (arrow) in a patient with Erdheim–Chester disease (ECD). **b**, Abdominal CT scan (axial view) showing peri-aortic (long arrow) and peri-renal (short arrow) infiltration in a patient with ECD. **c**, Abdominal CT scan (axial view) showing typical infiltration around both kidneys ('hairy kidneys') (arrows) in a patient with ECD. **d**, ^{99m}Tc bone scintigraphy showing bilateral uptake of tracer in the long bones of the lower limbs (arrows), a typical sign of ECD. **e**, Coronal view of a CT scan showing peri-renal infiltration in a patient with ECD (arrows). **f**, Retroperitoneal biopsy in a patient with ECD shows diffuse fibrosis, with a focal storiform pattern, and infiltration by foamy histiocytes (arrows) and multinucleated Touton giant cells (circle) (haematoxylin and eosin (H&E) stained, magnification ×20). **g**, Retroperitoneal biopsy in a patient with ECD shows fibrous bundles around small vessels (arrow) (H&E stained; magnification ×20). **h**, Retroperitoneal biopsy in a patient with ECD shows diffuse positivity for the histiocyte marker CD68 (anti-CD68KP1 antibody stained; magnification ×20). **i**, Coronal view of a CT scan showing massive and bilateral infiltration of the renal pelvis (arrows) extending to the calyces in a patient with Rosai–Dorfman disease (RDD). **j**, Peri-renal RDD lesion showing diffuse mononuclear cell infiltrates with signs of emperipolesis (circle) and scattered eosinophils (arrow) (H&E stained; magnification ×20). **k**, Skin biopsy in a patient with RDD displaying the presence of numerous S-100$^+$ cells (anti-S100 antibody stained; magnification ×10).

criteria. Second, the criteria exclude the possibility that a patient with IgG4-RD has an overlapping autoimmune disease such as systemic lupus erythematosus or ANCA-associated vasculitis (meaning that positivity for specific autoantibodies that suggests an alternative diagnosis represents an exclusion criterion), even though overlap cases have been extensively reported in the literature[70–72]. Third, typical disease lesions that usually show mild tissue and serum IgG4 responses (for example, retroperitoneal fibrosis, Riedel thyroiditis or mediastinal fibrosis) generally do not reach a sufficiently high score to be classified as IgG4-RD, when they occur in isolation[5,14,73–75]. This is indeed a conceptual issue: given the weakness of their IgG4 responses, it is probably incorrect to incorporate such lesions in IgG4-RD. Finally, the criteria place a substantial weight on histology and immunostaining, which becomes an issue, especially when biopsies are not available. These limitations notwithstanding, the ACR–EULAR 2019 criteria[65] represent the result of a large international effort and are flawed mainly because of the lack of diagnostic biomarkers and the intrinsic clinical variability of the disease.

In parallel with the American College of Rheumatology–European League Against Rheumatism criteria, in 2020 Japanese researchers revised the Comprehensive Diagnostic (RCD) criteria for IgG4-RD[76], updating a first set that was formulated in 2011[77]. The 2020 RCD criteria include three domains: clinical and radiological features, with demonstration of involvement of at least one organ; serological findings, namely elevated IgG4 levels; and histological diagnosis. A diagnosis of IgG4-RD is considered 'definite' when all three conditions are met, 'probable' when the first and third conditions are met and 'possible' when only the first two conditions are met (Fig. 4). Unlike the American College of Rheumatology–European League Against Rheumatism (classification criteria, which were developed using sizeable discovery and validation cohorts, the RCD criteria are based mainly on expert opinion. They have a high sensitivity and a low specificity (100% and 50%, respectively), and require validation[78].

Differential diagnosis

The differential diagnosis is one of the earliest and most important steps in the diagnostic approach to IgG4-RD, and some differential diagnoses (particularly autoimmune diseases) should be considered not only because they can mimic IgG4-RD but also because they can be associated with it.

Infections and neoplasms. First, it must be ruled out that lesions suspected as being IgG4-RD are infectious or neoplastic. Mycobacterial infections are indeed an important differential diagnosis, especially when they show systemic involvement (particularly pulmonary, lymph node, meningeal); retroperitoneal and/or prevertebral lesions mimicking retroperitoneal fibrosis might also be tubercular (for example, owing to an extension of Pott disease)[79] or triggered by mycobacterial infections occurring at remote sites such as the lung and mediastinal lymph nodes[80]. Syphilis can also cause a wide variety of clinical manifestations that mimic IgG4-RD, including cutaneous, neurological and aortic involvement. In general, chronic bacterial, fungal or viral infections should be ruled out in patients with suspected IgG4-RD.

The possibility of solid and haematological neoplasms also needs to be excluded as these conditions often mimic IgG4-RD or can coexist with it. Lymphoma is a common differential diagnosis[81]: it can be a systemic condition affecting nodal and extra-nodal sites, often presenting as mediastinal or retroperitoneal masses and showing polyclonal hypergammaglobulinaemia. However, even localized extranodal lymphoma (for example, orbital mass) could resemble IgG4-RD. Interestingly, patients with gain-of-function variants of *IKZF1* (encoding IKAROS) have been reported to develop both IgG4-RD and B cell malignancies[82].

Solid neoplasms must also be screened: metastatic diseases can mimic the systemic presentation of IgG4-RD, but obviously localized neoplasms must be excluded too. It has been consistently shown that the risk of both haematological and solid neoplasms is higher in patients with IgG4-RD than in the general population, with the frequency of malignancies peaking within the first 3 years after IgG4-RD diagnosis[81]. This pattern could be attributable to misdiagnosis or increased screening due to more frequent investigations, although it cannot be excluded that IgG4-RD per se predisposes to malignancies[81,83].

Histiocytoses and other proliferative disorders. Table 1 reports the frequencies of disease manifestations in IgG4-RD and other systemic inflammatory or non-malignant proliferative disorders[7,22,24,25,84–110]. Among these conditions, histiocytoses such as Erdheim–Chester disease (ECD) and Rosai–Dorfman disease (RDD; also known as sinus histiocytosis with massive lymphadenopathy, or Rosai–Dorfman–Destombes disease) are important differential diagnoses. ECD is a non-Langerhans cell histiocytosis that usually affects adults but only rarely affects children, and is characterized by tissue infiltration by foamy histiocytes[111,112]. ECD has a slowly progressive course and ranges from limited asymptomatic disease to systemic, aggressive forms. ECD is a perfect mimic of IgG4-RD (Table 1). It affects large and medium-sized vessels, often causing peri-arteritis; the so-called 'coated aorta' (that is, peri-vascular infiltration of the thoracoabdominal aorta) is an iconic feature of ECD (Fig. 5). Vascular involvement can also extend to the epi-aortic[113], coronary[114], renal[7] and mesenteric[115]

vessels and can be indistinguishable from that of IgG4-RD. One main differentiating feature of abdominal peri-aortitis in IgG4-RD versus ECD is its distribution, which is generally on the anterolateral sides of the aorta in IgG4-RD but presents as a thin circumferential thickening in ECD[116]. Retroperitoneal infiltration is also common in both conditions: in IgG4-RD, it commonly involves the peri-aorto-iliac space and causes medial deviation and/or stenosis of the lower third of the ureters[117], whereas in ECD it involves the peri-renal space (giving rise to the typical 'hairy kidney' appearance), the renal vessels, the proximal ureters and the renal sinuses[7] (Fig. 5). However, infiltration of the peri-renal space, including the pelvis and the vascular peduncle, can also occur in IgG4-RD (Fig. 3). Other features shared by ECD and IgG4-RD are mesenteric, mediastinal, pleural and peri-cardial involvement, retro-orbital masses, paranasal sinus involvement, pachymeningitis and hypothalamic–pituitary involvement[111,118]. Altogether, these overlapping features make differential diagnosis particularly difficult. A distinguishing feature of ECD (and absent in IgG4-RD) is long-bone infiltration, which is symmetrical, mainly involves the femurs and tibia, and causes osteosclerotic lesions that have a high uptake of tracer on ^{99m}Tc bone scintigraphy and FDG-PET (Fig. 5). The final diagnosis of ECD is histological (see below, paragraph on biopsy), and molecular analysis of the pathological tissue is required to investigate underlying somatic mutations, which are found in ~90% of patients; somatic mutations have not been reported in IgG4-RD, although specific studies are probably lacking[119,120]. IgG4 responses have not been systematically investigated in ECD, but high IgG4 levels and prominent IgG4$^+$ plasma-cell infiltration have been reported in case reports and case series of ECD[5,63,121].

RDD is an additional, important differential diagnosis. It is also a systemic histiocytosis but is clinically and histologically distinct from ECD, although ECD–RDD overlap is recognized[122]. Somatic mutations are found in <50% of RDD cases[123]. RDD usually affects the lymph nodes but the involvement of extra-nodal sites, such as meninges, upper respiratory tract, orbit, bone and salivary glands, is common[123,124]. Retroperitoneal peri-renal infiltration usually has a predilection for the renal hila and the proximal ureters, without peri-capsular involvement[8]. RDD can be misdiagnosed as IgG4-RD owing to the frequent detection of polyclonal hypergammaglobulinaemia with increased IgG4 levels, and of numerous IgG4$^+$ plasma cells in the affected tissues[125,126]. The diagnosis of RDD is essentially histological, with demonstration of tissue infiltration by histiocytes often showing signs of emperipolesis (Fig. 5), a non-destructive form of phagocytosis.

iMCD is a lymphoproliferative disorder of unknown aetiology hallmarked by lymphadenopathies, without evidence of human herpesvirus 8 infection. Its plasmacytic subtype, iMCD-idiopathic plasmacytic lymphadenopathy (iMCD-IPL), presents clinically with diffuse lymphadenopathy and an acute-phase reaction characterized by polyclonal hypergammaglobulinaemia (frequently with high IgG4 levels), high C-reactive protein levels and thrombocytosis. Histologically, iMCD-IPL very frequently shows nodal infiltration by IgG4$^+$ plasma cells and can mimic IgG4-RD lymphadenopathy[6].

Systemic vasculitides and other immune-mediated diseases. Large-vessel vasculitis (LVV) syndromes, namely GCA and Takayasu arteritis, share with IgG4-RD tropism for large arteries (Table 1). Unlike IgG4-RD, GCA usually has an abrupt presentation with cranial symptoms and sudden visual loss, and requires urgent management. However, a subset of individuals with GCA that have predominant large-vessel (aorta and epi-aortic arteries) involvement and systemic symptoms is well recognized[127]. If temporal artery biopsy is not diagnostic, as often occurs with the large-vessel phenotype of GCA, magnetic resonance angiography, CT angiography and FDG-PET are crucial in the differentiation between GCA and IgG4-RD, with GCA showing more intense and diffuse vascular involvement and a higher frequency of carotid, subclavian and axillary artery involvement than IgG4-RD[128]. Interestingly, IgG4-RD can also involve the temporal arteries, although in most cases it is peri-arteritis and not transmural arteritis, as is commonly observed in GCA[129].

Takayasu arteritis is another LVV syndrome that affects younger individuals. It usually involves the thoracic aorta and the epi-aortic arteries, but also other vessels including the abdominal aorta and its branches. The onset of Takayasu arteritis is usually insidious and therefore similar to that of IgG4-RD; unlike IgG4-RD, however, it commonly causes severe stenosis of the large vessels, especially the epi-aortic and renal arteries[130]. Takayasu arteritis should be considered in patients with suspected IgG4-RD, especially when they have isolated peri-aortitis of the thoracic and/or abdominal aorta[13]. Ureteral and/or inferior vena-cava encasement by abdominal peri-aortitis distinguishes between IgG4-RD and LVV[11].

Small-vessel vasculitides, particularly granulomatous forms of ANCA-associated vasculitis such as granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA) can also mimic some manifestations of IgG4-RD. Notably, both syndromes involve increased serum levels of IgG4 in a remarkable proportion of cases (~80% of patients with active EGPA and ~30% with active GPA)[64]. Although differentiating IgG4-RD from EGPA might be more straightforward, as EGPA is hallmarked by marked eosinophilia, onset in adult years, severe asthma and nasal polyposis[131], GPA has a broader overlap with IgG4-RD; GPA can indeed involve the orbit, pachymeninges and pre-vertebral areas and occasionally cause peri-aortitis or mediastinitis[132] (Table 1). However, GPA usually has a faster course than IgG4-RD, with aggressive manifestations such as rapidly progressive glomerulonephritis, alveolar haemorrhage or sensorimotor peripheral neuropathy[132]. ANCA positivity is generally considered an exclusion criterion for the classification of a disease as IgG4-RD. However, it must also be emphasized that, despite their profound histological and pathophysiological differences, ANCA-associated vasculitides and IgG4-RD might coexist[70,133]. The exact nosology of these cases remains to be determined but it is important to be aware that patients with such composite phenotypes can be encountered in clinical practice.

Other granulomatous disorders, such as sarcoidosis (Table 1), can also overlap with systemic IgG4-RD manifestations, such as lymphadenopathy, lung involvement, tubulo-interstitial nephritis and orbital lesions[98]. Histology is usually essential for the differential diagnosis. Serum levels of chitotriosidase and angiotensin-converting enzyme can help in the differential diagnosis, being elevated in most patients with active sarcoidosis[134], but their specificity is low and they have not been tested in IgG4-RD. IgG4-RD has also been described, although anecdotally, in patients with a previous diagnosis of sarcoidosis[72].

Finally, other immune-mediated or haematological conditions (such as Sjögren disease, amyloidosis and hypereosinophilic syndrome) must be considered in the differential diagnosis, mainly because they can also be systemic and the sites they affect are shared with IgG4-RD[62]. However, the diagnostic approaches to these conditions are usually well defined and IgG4 responses are generally weak.

Histopathology

IgG4-RD has key histopathological features that are quite uniformly present in all affected organs: dense lymphoplasmacytic infiltrates

comprising T and B cells and plasma cells, often forming germinal centres, along with scattered eosinophils; fibrosis, usually with a storiform appearance; and phlebitis, which may or may not be obliterative. Hyaline rings surrounding small arteries or capillaries and peri-vascular fibrosis of medium-sized and large arteries is also common[135] (Fig. 1). Histopathological features considered to be against a diagnosis of IgG4-RD include granulomas, necrosis, neutrophil-dominant inflammation and, obviously, lesions compatible with infections or malignancies[65]. Immunostaining for IgG4 and IgG is mandatory; a ratio of $IgG4^+$ to IgG^+ plasma cells of >40% is considered suggestive of IgG4-RD, although it is neither sensitive nor specific, and can vary from organ to organ[135].

Histiocytoses mimic IgG4-RD because of their clinical and histopathological aspects and their tendency to form neoplasms of various sizes in a great variety of organs. Similar to IgG4-RD, in which the presence of $IgG4^+$ plasma cells is key to the diagnosis, histiocytoses are classified on the basis of a particular cell having a pivotal role in the composition of the lesion. And just like in IgG4-RD, the lesional cells of the histiocytoses represent only a small proportion of the total number of cells present in the lesions, and are surrounded and sometimes overshadowed by a non-specific, inflammatory mononuclear cell infiltrate[136,137]. For these reasons these entities present a diagnostic challenge for the pathologist. Especially with biopsy-obtained specimens, via which only a small sample of the lesion is obtained, the first impression is that of a non-specific inflammatory lesion. In contrast to IgG4-RD, the lesional cells in histiocytoses are considered to be variants of the commonly occurring histiocytes due to mutations of genes involved in the MAP kinase cell-signalling pathway. Whereas histiocytoses are characterized by positivity for the histiocyte-macrophage marker CD68, the lesional cells can be identified with additional immunohistochemistry testing, mainly for S100 and CD1a expression: ECD involves $CD1a^-$ $S100^{+/-}$ histiocytes, RDD mainly $CD1a^-$ $S100^+$ cells and Langerhans cell histiocytosis $CD1a^+$ $S\text{-}100^+$ cells[137] (Fig. 5).

Overlapping histological features between ECD and IgG4-RD include fibrosis, which sometimes has a storiform pattern and surrounds small vessels, and chronic lymphoplasmacytic infiltrates (Fig. 5). When detected, foamy histiocytes and multinucleated Touton giant cells indicate ECD. The histopathological differentiation between RDD and IgG4-RD can also be challenging: hallmark features of RDD such as emperipolesis might be absent in RDD biopsy-obtained specimens; similarly, storiform fibrosis and obliterative phlebitis are rare in IgG4-RD lymphadenopathy. As stated above, $IgG4^+$ plasma cells can be abundant in RDD[116]. For both ECD and RDD, molecular analysis of the lesional tissue can suggest the diagnosis of histiocytosis if disease-related somatic mutations (for example, in *BRAF*, *NRAS*, *KRAS* or *MAP2K1*) are detected[120,123].

iMCD-IPL also poses histopathological challenges. Both iMCD-IPL and IgG4-RD are marked by prominent $IgG4^+$ responses in the lymph nodes. Histologically, the background of iMCD-IPL is often different from that of IgG4-RD lymphadenopathy, with hyperplastic germinal centres and sheet-like proliferation of mature plasma cells in expanded interfollicular areas[6].

Histological examination is crucial in the differentiation between IgG4-RD and granulomatous disorders such as sarcoidosis, GPA or EGPA, as the demonstration of granulomatous lesions or, in the case of vasculitic syndromes, fibrinoid necrosis are strong arguments against IgG4-RD[135].

The role of imaging and serological findings

Radiological assessment is also important for the diagnosis and staging of IgG4-RD, is guided by clinical manifestations and differs on the basis of the organs involved. Ultrasonography, CT and MRI all contribute to the assessment of most disease manifestations. In whole-body imaging, an important role is also played by ^{18}F-FDG-PET, which detects metabolically active lesions[138,139] (Fig. 2). ^{18}F-FDG-PET could also have prognostic relevance for specific disease manifestations (for example, retroperitoneal fibrosis), as ^{18}F-FDG-avid lesions seem to be more sensitive to immunosuppressive therapies than ^{18}F-FDG-negative lesions[15,140]. Whole-body CT or MRI and ^{18}F-FDG-PET should be performed in all patients for a thorough initial assessment, whereas during follow-up, CT and MRI should focus only on the affected sites (for example, brain MRI for cerebral or meningeal lesions, and high-resolution chest CT for lung or pleural lesions); ultrasonography can be a reliable, non-invasive and cheap technique for monitoring complications such as hydronephrosis due to retroperitoneal fibrosis. The optimal imaging strategy, however, varies from patient to patient.

Among laboratory parameters, a high serum level of IgG4 is the most distinctive laboratory finding of IgG4-RD. However, as reported above, an increase in serum IgG4 level can also be found in a wide spectrum of inflammatory, allergic, infectious and proliferative disorders[5,141,142]. The specificity of IgG4 increases when its levels are more than twofold the upper limit of normal, and is high with levels more than fivefold the upper limit of normal. Serum IgG4 levels swiftly decline after glucocorticoid and/or immunosuppressive therapy; high levels at baseline are generally associated with multi-system involvement[5], although the disease phenotype also matters[24] (Supplementary Table 1). Other serological markers that correlate with disease extent and activity include serum levels of IgG1, IgE and complement; a reduction in serum levels of C3 and C4 is associated with multisystem disease and particularly with lymph-node, pulmonary, renal and pancreato-biliary involvement[143].

Treatment

General principles of treatment

Given the slowly progressive course of IgG4-RD, urgent treatment is seldom required, with the exception of specific situations that might necessitate interventional procedures (for example, ureteral or biliary stent placement for the treatment of ureteral obstruction or biliary tract stenosis, respectively) (Fig. 6). Prompt treatment is needed for symptomatic patients and for asymptomatic patients with evidence of progressive disease in vital organs. Individuals with asymptomatic, limited disease warrant careful monitoring[144]. Current therapies are aimed at curbing the inflammatory process, eventually preventing tissue fibrosis. Glucocorticoids are the mainstay of therapy. Nevertheless, the disease frequently relapses, and long-term glucocorticoid maintenance is associated with severe adverse effects, especially considering that IgG4-RD usually affects elderly individuals. Thus, interest is growing in glucocorticoid-sparing agents. The overall treatment approach should take into account the chronic–relapsing course of the disease, comorbidities and potential treatment-related adverse effects. Courses of glucocorticoids of 9–12 months are usually used; whether glucocorticoids can be tapered to withdrawal or maintained at low doses is debated. The introduction of B cell-targeting therapies will most likely obviate the long-term use of glucocorticoids.

Glucocorticoids and glucocorticoid-sparing agents

Medium-to-high-dose glucocorticoids (~0.6 mg/kg per day of prednisone or equivalent) are recommended as first-line therapy for IgG4-RD if there are no contraindications, although the choice of this initial dose is based mainly on expert opinion (Fig. 6). A significant

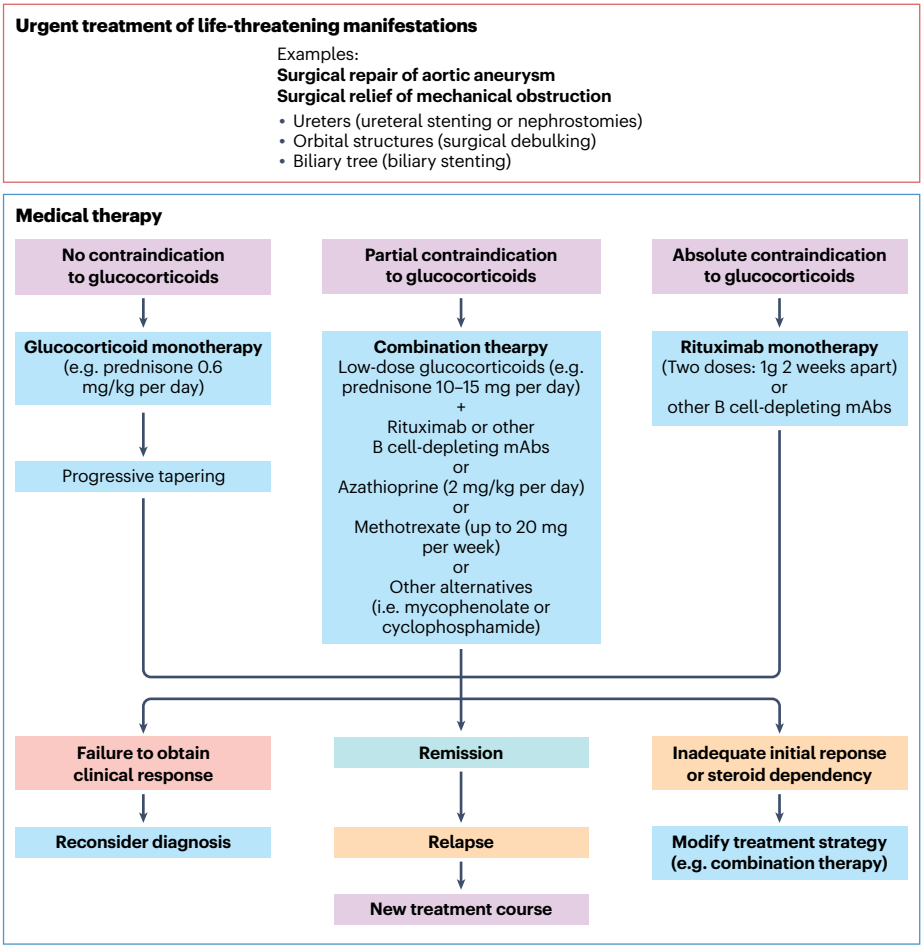

Fig. 6 | Treatment algorithm for IgG4-related disease. The flowchart provides a structured approach to the treatment of IgG4-related disease, guiding therapeutic decisions on the basis of clinical presentation and response to therapy. Initial treatment depends on the urgency of the condition, which might require prompt surgical intervention, and on contraindications to glucocorticoid use. Patients without contraindications to glucocorticoids typically receive glucocorticoid monotherapy, whereas those with partial or absolute contraindications are managed with steroid-sparing agents or B cell-depleting monoclonal antibodies (mAbs). In cases of inadequate response, relapse, or steroid dependency, treatment modification is recommended.

improvement in symptoms is usually observed within days to weeks of the initiation of glucocorticoid therapy, whereas the reduction or normalization of radiological and serological findings could take weeks to months. Lack of response to glucocorticoids is an exclusion criterion for the classification of IgG4-RD according to the ACR–EULAR criteria[65] (Fig. 4), which underscores the importance of glucocorticoid sensitivity in IgG4-RD. Both retrospective and prospective studies report response rates to glucocorticoid that exceed 85%; refractoriness to glucocorticoids, which therefore occurs in <15% of the patients, is thought to be attributable to predominantly fibrotic lesions. Glucocorticoids are also effective in reducing relapse rates when used as maintenance therapy[145,146]. In a randomized controlled trial, prednisone was superior to tamoxifen (which was initially thought to be a valid alternative to glucocorticoids because of its presumed immunomodulatory and antifibrotic effects) for the maintenance of remission in patients with idiopathic retroperitoneal fibrosis[147]. With the aim of limiting the cumulative dose of glucocorticoids, several traditional immunosuppressants (such as azathioprine and methotrexate) have been investigated for the treatment of IgG4-RD. Evidence comes from retrospective studies and proof of their efficacy in inducing and maintaining remission is limited[148–150]. Nonetheless, observational studies have reported that the addition of other steroid-sparing agents (cyclophosphamide and mycophenolate mofetil) is associated with a lower relapse rate when compared with glucocorticoid monotherapy[151].

Emerging therapies

B cell-targeting agents. Since the publication of a first case report in 2008 (ref. 152), retrospective and prospective studies have demonstrated that rituximab and other anti-CD20 monoclonal antibodies can induce remission in up to 80% of cases of IgG4-RD. Data from a meta-analysis indicate a lower relapse rate following such treatments when compared with other immunosuppressants[153]. The mounting evidence regarding the pathogenic role of B cells in IgG4-RD strengthens the rationale for the use of anti-CD20 and other B cell-targeting agents.

Rituximab is frequently used in combination with glucocorticoids as first-line treatment, especially in patients with multisystemic disease and/or vital organ involvement and in patients who cannot tolerate medium-to-high doses of glucocorticoids because of comorbidities (such as diabetes mellitus, obesity or depression) (Fig. 6). Patients who have an inadequate response to glucocorticoids, who relapse after glucocorticoid-induced remission or who cannot satisfactorily reduce their glucocorticoid dose are also commonly prescribed rituximab. Finally, rituximab can also be used as monotherapy for remission induction in patients with absolute contraindications to glucocorticoids, and is a valid option for maintenance therapy as well[154–156]. For remission induction, rituximab is commonly used at a dose of 1 g intravenously every 15 days for a total of two doses, whereas maintenance regimens and dosing strategies are heterogeneous; observational studies demonstrated that systematic rituximab infusions can improve relapse-free

survival[154]. Despite its recognized efficacy in IgG4-RD, rituximab has not been tested in randomized controlled trials and is used off label.

The role of serum levels of IgG4 in predicting response to rituximab or other therapies is controversial. For at least some manifestations (for example, retroperitoneal fibrosis) rituximab is also effective in patients with no elevation in serum IgG4 levels or histological evidence of IgG4$^+$ plasma-cell infiltration[5,157].

Beyond rituximab, the most promising drugs currently under investigation are inebilizumab and obexelimab. Inebilizumab, a B cell-depleting anti-CD19 monoclonal antibody, has been evaluated in MITIGATE, a phase III randomized placebo-controlled trial that enrolled adults who were receiving glucocorticoid treatment for a current IgG4-RD flare[158]. Inebilizumab (at a dose of 300 mg) or placebo was administered by intravenous infusion on days 1 and 15 and at week 26 and identical glucocorticoid tapers were used in both treatment groups (with the aim of withdrawal by week 8). The results, published in late 2024, indicate that the primary end point (time to first flare) was met: inebilizumab reduced the risk of IgG4-RD flare by 87% compared with placebo over 52 weeks. Serious adverse events during the treatment period were seen in 18% and 9% of the patients who received inebilizumab and placebo, respectively[158].

Obexelimab is a humanized monoclonal antibody that ligates CD19 with its Fab portion, whereas its Fc portion is engineered to engage the inhibitory FcγRIIb receptor. The co-ligation of CD19 and FcγRIIb leads to inhibition of B cells without inducing B cell depletion. Encouraging results from a phase II open-label study[159] led to a phase III randomized placebo-controlled trial, which is currently enrolling (NCT04540497).

Other B cell-targeting drugs currently under investigation as potential treatments for IgG4-RD include the Bruton tyrosine kinase inhibitors rilzabrutinib (NCT04520451) and zanubrutinib (NCT04602598); telitacicept, a recombinant fusion protein comprising the extra-cellular domain of T cell activator and calcium-modulating ligand interactor, and capable of neutralizing both BAFF and APRIL[160]; and belimumab (NCT04660565), a recombinant monoclonal antibody against soluble BAFF, the levels of which are increased in active IgG4-RD[161].

T cell-targeting agents. T cell activation also represents a promising target in IgG4-RD. Abatacept is a synthetic analogue of cytotoxic T lymphocyte antigen 4 (CTLA4) that binds CD80–CD86 and prevents T cell activation by competing with the costimulatory molecule CD28 expressed by T cells. In a prospective open-label study that included ten patients with active IgG4-RD (seven of whom were treated with abatacept alone), abatacept demonstrated efficacy in five patients[162]. The monoclonal antibody elotuzumab, which targets SLAMF7, was investigated in a prospective trial that was terminated early because of a lack of efficacy (NCT04918147).

Cytokine-targeting agents. Levels of several cytokines correlate with IgG4-RD activity and their expression is enhanced in affected tissues; thus, these cytokines represent potential therapeutic targets. Eosinophils are frequently observed in IgG4-RD lesions and blood concentrations of these cells are increased in one-third of cases of IgG4-RD; however, the use of mepolizumab or benralizumab, which target the IL-5–IL-5 receptor axis, thus hampering the maturation, activation and survival of eosinophils, proved ineffective[163]. IL-4 and IL-13 were both described to be involved in IgG4-RD and the development of tissue fibrosis; nevertheless, blocking the IL-4–IL-13 receptor pathway with dupilumab in IgG4-RD led to conflicting results[164,165]. Janus kinase (JAK) inhibitors represent a promising alternative: tofacitinib proved effective in inducing either complete or partial responses in two patients with IgG4-RD and two patients with idiopathic retroperitoneal fibrosis[166]. Two trials of JAK inhibitors for the treatment of IgG4-RD are ongoing, investigating tofacitinib (NCT05625581) and baricitinib (NCT05781516), respectively.

Conclusions

IgG4-RD is a complex and multifaceted fibro-inflammatory disease and its clinical phenotype ranges from organ-limited to disseminated multisystemic forms. The lack of disease-specific biomarkers and diagnostic criteria often pose challenges for the diagnostic approach. After infections and malignancies have been ruled out, a number of systemic conditions, mainly histiocytoses, vasculitis, granulomatous and lymphoproliferative diseases, must be considered in the differential diagnosis, as their clinical and histological presentations can overlap with that of IgG4-RD and also because they might harbour pronounced IgG4 responses. The spectrum of IgG4-RD also includes forms with mild IgG4$^+$ plasma-cell infiltration and normal serum levels of IgG4, the nosology of which is still uncertain.

Glucocorticoids and B cell-depleting agents induce clinical responses in the vast majority of cases of IgG4-RD. However, the most appropriate remission-induction and remission-maintenance strategies still need to be established. Advances in the comprehension of the pathogenesis of IgG4-RD have prompted the development of novel therapies, mainly targeting B cells, T cells and cytokines, which are being tested in clinical trials. Future areas of scientific research should explore diagnostic biomarkers, disease-specific and organ-specific prognostic factors, and cost-effective treatment strategies, the development of which requires the involvement of patients and the analysis of patient-reported outcomes.

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Review article

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Restoring articular cartilage: insights from structure, composition and development

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Abstract

Articular cartilage can withstand substantial compressive and shear forces within the joint and also reduces friction during motion. The exceptional mechanical properties of articular cartilage stem from its highly organized extracellular matrix (ECM). The ECM is composed mainly of collagen type II and is pivotal in conferring mechanical durability to the tissue within its proteoglycan-rich matrix. Articular cartilage is prone to injury and degeneration, and current treatments often fail to restore the mechanical function of this tissue. A key challenge is replicating the intricate collagen–proteoglycan network, which is essential for the long-lasting restoration and mechanical durability of the tissue. Understanding articular cartilage development, which arises between late embryonic and early juvenile development, is vital for the creation of durable therapeutic strategies. The development of the articular ECM involves the biosynthesis, fibrillogenesis and self-assembly of the collagen type II network, which, along with proteoglycans and minor ECM components, shapes the architecture of adult articular cartilage. A deeper understanding of these processes could inform biomaterial-based therapies aimed at improving therapeutic outcomes. Emerging biofabrication technologies offer new opportunities to integrate developmental principles into the creation of durable articular cartilage implants. Bridging fundamental biology with innovative engineering offers novel approaches to generating more-durable 3D implants for articular cartilage restoration.

Sections

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Key points

- Articular cartilage is a highly anisotropic tissue that is characterized by depth-dependent collagen fibre orientation, which provides the tissue with its unique biomechanical properties.
- The collagen architecture of the articular cartilage forms during late fetal and postnatal development in response to biomechanical stimuli and tissue growth.
- Collagen interacts with the surrounding extracellular matrix (ECM), including aggrecan, by limiting the water-swelling pressure caused by negatively charged glycosaminoglycans, thereby enhancing cartilage resilience.
- Small proteoglycans and (glyco)proteins promote tissue cohesion and stability by regulating fibrillogenesis and connecting various ECM components together.
- Achieving native-like collagen anisotropy in engineered cartilage remains a challenge, as current scaffolds cannot replicate the depth-dependent alignment of collagen and its ECM interactions.
- Insights gained from cartilage development and its anisotropic organization could inspire novel strategies for achieving long-term cartilage regeneration.

Introduction

Articular cartilage is a highly specialized connective tissue located in the synovial joint cavities lining the end of the epiphyses. The tissue consists of a thin hyaline cartilage layer (~0.1–2.5 mm, depending on location, age, sex and/or species[1–3]), which is important for the biomechanical function of the joint. The unique poroelastic properties of the tissue facilitate load transmission to the underlying bone and reduce friction to enable continuous smooth and pain-free movement[4]; however, the limited regenerative capacity of articular cartilage represents a major challenge for repair after injury or in disease. Therefore, understanding the composition and development of articular cartilage is crucial to improving therapeutic strategies.

The extracellular matrix (ECM) provides exceptional biomechanical support through its typical anisotropic structure and composition[5]. Collagen type II is the main fibrillar protein in articular cartilage[6], it forms heterotypic fibres with collagen type XI (inside the fibre) and type IX (on the fibre surface) and interacts with numerous other proteins and small molecules[7,8] In adult articular cartilage, these collagen fibrils run parallel to the articular joint in the superficial cartilage and curve perpendicular to the surface with depth, forming an arcade-like structure (also known as "Benninghoff arcades")[9]. Proteoglycans, predominantly aggrecan, are the second most abundant component in the articular ECM[10]. Aggrecan molecules are glycosylated with glycosaminoglycans (GAGs), mainly chondroitin sulphate and keratan sulphate (which are two repeating disaccharide polymers). Aggrecan molecules are negatively charged, which creates high osmotic potentials that draw water into the tissue, resulting in a gel-like composition with shock-absorbing properties[11]. The collagen fibre arcade-like structure limits aggrecan-induced swelling, which generates high swelling pressures that create the unique biomechanical property of cartilage that enables this tissue to withstand daily shear forces and impact loading[12].

The regenerative capacity of articular cartilage is limited owing to it being avascular, aneural and dependent on nutrient diffusion from the synovial fluid[13]. Furthermore, although resident chondrocytes synthesize and maintain the ECM in their proximity[14], the overall density of these cells throughout the cartilage tissue is low (~4.2×10^6 cells per cm^3)[15]. Thus, ECM degradation and articular cartilage injuries can cause pain, impaired mobility and a carry a high risk of developing into osteoarthritis (OA)[16].

Collagen structure forms during late fetal and early juvenile development, with minimal turnover in the adult tissue[17]. After articular cartilage injury, fibrotic scar tissue is deposited and mainly consists of mechanically inferior collagen type I[18]. Current treatments, including autograft implants[19], cell-based (stem cell and chondrocyte transplantation)[20] and biomaterial-based (often collagen type I scaffolds)[21] therapies, fail to effectively restore the original ECM architecture in the long term, highlighting the need for new approaches to restoring tissue function[22]. Meanwhile, tissue engineering and 3D bioprinting technologies offer great promise for providing patients with living implants that can repair chondral and osteochondral defects[23,24]; however, although such 3D-printed implants might show potential in the short term, they do not restore the collagen architecture and the aggrecan gradient, meaning that the implant will fail once the initial load-supporting materials have degraded[25]. The lack of long-term therapies highlights the need for a deeper understanding of the mechanisms that govern collagen alignment during embryonic and postnatal development. The cues that guide ECM composition and reorganization could provide valuable insights for novel biomimetic therapies that are capable of reproducing the long-term biomechanical function of the articular cartilage[25].

In this Review, we explore the basic composition, structure and development of articular cartilage, with a particular focus on how the anisotropic composition and structure of the collagen and proteoglycan networks are shaped. We highlight potential strategies for directing collagen alignment in engineered tissues and aim to explore how novel biomimetic therapies could reproduce articular tissue anisotropy. Finally, we examine novel biofabrication technologies that hold promise for directing cartilage ECM alignment, with potential therapeutic applications for chondral and/or osteochondral implant engineering.

The anisotropic nature of articular cartilage

Articular cartilage is a highly anisotropic tissue; at birth, the ECM is homogenous and predominantly isotropic, but during maturation, the ECM composition and structure of non-calcified articular cartilage develops into different zones[9]. This anisotropy is essential for the tissue to withstand the complex mechanical forces in the joints.

Collagen and proteoglycans in articular cartilage

On the basis of the orientation of collagen fibres, the zones of articular cartilage can be classified into the superficial zone (also known as the tangential zone), the middle zone (also known as the transitional zone) and the deep zone (also known as the radial zone[26] (Fig. 1). The superficial zone is relatively thin[27], with a low proteoglycan content and fine collagen fibres aligned parallel to the surface, which provide resistance to both tensile and shear forces[28]. The collagen fibres parallel to the articular surface not only exhibit depth-dependent organization but also a distinct arrangement across the entire articulating surface (known as split-line orientation patterns)[29]. This organization aligns

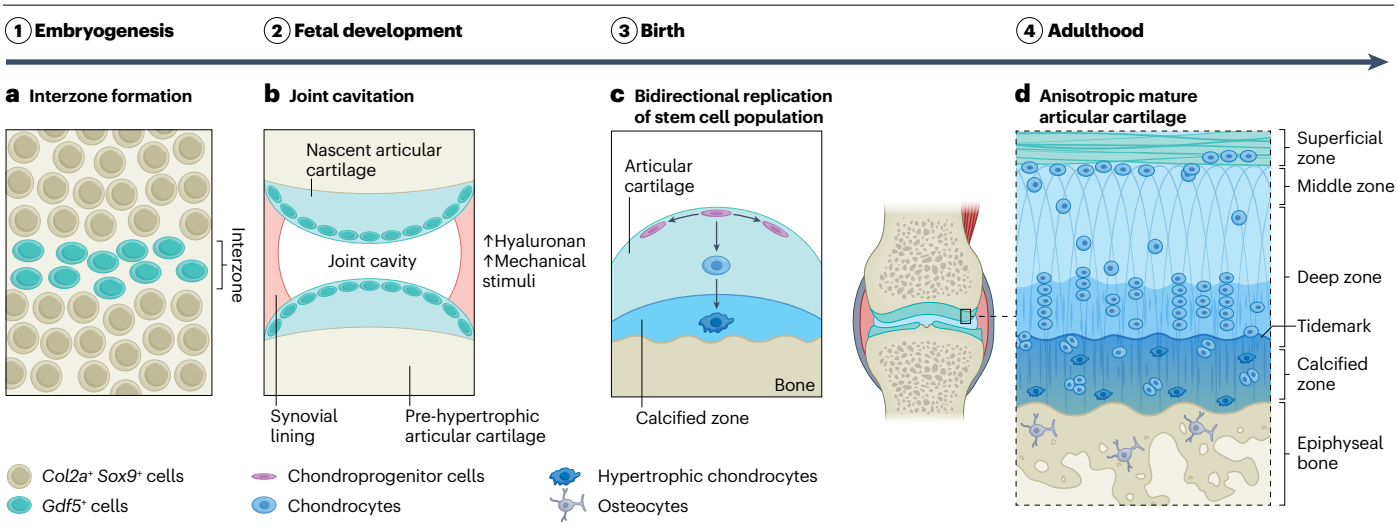

Fig. 1 | Development of knee articular cartilage from embryogenesis to adulthood. a, Interzone formation in the nascent limb occurs when flattened, condensed *Gdf5*$^+$ (ref. 61) interzone cells emerge within pre-cartilage tissue that is composed of *Col2a1*$^+$ *Sox9*$^+$ cells[58,59]. **b**, Joint cavitation occurs when increased hyaluronan synthesis drives fluid influx, with mechanical stimuli and muscle contractions contributing to cavitation[62]. **c**, Chondroprogenitor cells at the surface of postnatal articular cartilage undergo bidirectional replication, producing a columnar progeny of chondrocytes and maintaining a population of self-renewing chondroprogenitors[26,56,67,94]. **d**, After maturation, the tissue exhibits zonal organization: the superficial zone has collagen fibres oriented parallel to the surface, the middle zone exhibits an isotropic fibre arrangement and the deep zone contains vertically aligned collagen fibres, forming an arcade-like structure[9]. The innermost calcified zone, containing hypertrophic chondrocytes, anchors the cartilage to the subchondral bone, which hosts various bone cells, including osteocytes[66].

with the maximum tensile stresses experienced by the joint surface during motion and weight bearing; high tensile strength occurs in a direction that aligns with these fibres[30]. Consequently, the articular cartilage surface also has anisotropic properties; for example, split-line orientation patterns are more consistent in areas that experience more contact than those that have less contact, which enhances the ability of the tissue to withstand and distribute mechanical loads across the joint surface[30].

The superficial zone is rich in lubricin (also known as proteoglycan-4), a proteoglycan that contributes to articular-surface lubrication (leading to static and kinetic friction coefficients of 0.01 and 0.003, respectively[31]). Chondrocytes near the articular surface have a flattened morphology (Fig. 1); articular cartilage progenitor cells (ACPCs) are also found in this area[32–34]. These multipotent chondroprogenitor cells make up 0.1–1% of the total cells in cartilage and are present in both young and adult cartilage[35–39]. In response to in vivo cartilage injury, ACPCs migrate to the damaged site, suggesting a potential role for these cells in tissue homeostasis and repair[40]. The high proliferative capacity of ACPCs and their ability to undergo chondrogenic differentiation[41–43] make them promising candidates for therapeutic applications in neo-cartilage formation[39].

In the middle zone, the orientation of collagen fibres is random, whereas in the deep zone — the thickest layer — fibres are aligned perpendicular to the surface (Fig. 1). Compared with other zones, the deep zone contains the highest proteoglycan content and the thickest collagen fibres[44], which anchor into the subchondral bone[45]. Oval chondrocytes are distributed throughout this zone and are arranged in vertical columns[33] (Fig. 1). The deepest layer is the calcified cartilage, which anchors to the underlying epiphyseal bone. Here, chondrocytes are sparse and hypertrophic[46], and collagen type X is abundant[47]. This layered organization has remained preserved across the evolution of terrestrial mammals[27,48,49].

The ECM is divided into three regions on the basis of chondrocyte proximity: the pericellular matrix (PCM, 2–4 µm), the territorial matrix and the interterritorial matrix. The PCM is in direct contact with chondrocytes and differs from the territorial and interterritorial areas in structure, function and composition. The PCM controls chondrocyte phenotypes, acts as a growth factor reservoir and is important for mechanotransduction[50]. The major components of the PCM are perlecan[51], collagen type VI[52] and fibronectin[53]. Collagen type VI forms a beaded microfibrillar network that anchors the chondrocytes to the surrounding ECM and mediates cell–matrix interactions via integrin cell receptors[54]. In the PCM, collagen fibres are oriented in a basket-like pattern around the chondrocytes[55]. The combination of the PCM and chondrocytes is referred to as the chondron[44,50]. The chondron is immediately surrounded by the territorial matrix, which is rich in proteoglycans, and the interterritorial matrix, which comprises the majority of the ECM.

The bottom–up development of articular cartilage

During embryonic development, articular cartilage formation begins with the establishment of the synovial joints[56]. Lineage-tracing studies in mice have provided insights into this complex process. Joint establishment starts with the formation of the interzone, a region that initially consists of Col2a1$^+$ Sox9$^+$ cells that are recruited from the mesenchymal condensation of the developing limb bud[57]. These mesenchymal progenitors flatten and compact to settle in the future joint[58] (Fig. 1a). As development continues, the interzone is populated by cells that express *Gdf5*, including cells that later migrate into the developing limb[59]. *Gdf5*$^+$ cells are responsible for forming articular

cartilage and other joint structures (such as the ligament and the meniscus). Several hypotheses have been proposed to explain how *Gdf5*-expressing cells give rise to tissue divergence[60]. This process is followed by joint cavitation[61]. Joint cavities form from fluid influx that is induced by the upregulation of hyaluronan synthesis[62], which causes swelling pressure that separates the interzone into two opposing tissues (Fig. 1b). Mechanical stimuli, via fetal muscle contraction, also has a role in joint cavity formation[63]. Interzone and joint cavitation are regulated by numerous factors and signalling pathways[58]. After the synovial joint space is formed, the articular ends start a morphogenesis process, in which early cartilage shape, organization and structure are established[64].

Chondroprogenitor cells that are present at the surface of the nascent joint contribute to the development of all the layers of articular cartilage and supply rapidly proliferating daughter cells that move down vertically to build the ECM (Fig. 1c). These chondroprogenitor cells express *Prg4* and are hypothesized to be descendants of interzone cells that downregulate *Gdf5* expression after birth[65]. The descendant chondrocytes will migrate further and undergo hypertrophy and apoptosis after building an ECM that is invaded by blood vessels and various bone cells, creating calcified bone tissue (known as the secondary ossification centre). The hypertrophic chondrocytes that do not die can transition into osteogenic cells[66].

Some daughter cells will halt hypertrophy as they migrate downwards and retain a chondrocyte phenotype, forming a layer of non-ossified cartilage[67]. The tidemark is the boundary between the non-calcified and calcified zones (Fig. 1d). Thereafter, a bottom–up growth of articular cartilage tissue occurs above the calcified layer, which is gradually built up by chondrocyte proliferation and ECM deposition[58,68,69]. During late embryonic and postnatal tissue growth, articular cartilage undergoes further morphogenesis, marked by the realignment of the ECM. Collagen fibres are initially more isotropic but eventually become perpendicular to the surface in the deep zone and parallel to the surface in the superficial zone (Fig. 1d).

Collagen type II fibres in the articular cartilage matrix

Collagen is present in the articular ECM in the form of heterotypic fibres; typically, collagen type II is packed together with collagen type XI, creating tightly packed, resilient networks[6]. This process is facilitated by the presence of other proteins that interact with these fibres, such as collagen type IX (on the fibre surface)[70] and small leucine-rich proteoglycans[71], which not only facilitate assembly but also regulate fibre diameter. For simplification, hereafter, we refer to heterotypic cartilage collagen fibres as collagen type II fibres. Collagen biosynthesis and fibrillogenesis contribute to the growth and integrity of the tissue[72]. Therefore, to elucidate how this complex architecture is achieved in the mature tissue, it is important to understand the key elements of collagen synthesis, its release into the ECM and the process of fibrillogenesis into the existing network.

Collagen biosynthesis

Collagen type II biosynthesis begins with the transcription of the *COL2A1* gene into two messenger RNA (mRNA) isoforms (IIA and IIB) by alternative splicing. Chondroprogenitor cells synthesize collagen IIA and mature differentiated chondrocytes synthesize collagen IIB[73]. Collagen IIA contains an additional exon, leading to a cysteine-rich domain in the N-pro-peptide region, which interacts with TGF-β and BMPs. Collagen IIA is expressed during the early stages of cartilage development[74]. Collagen IIB is synthesized by adult chondrocytes and is the major form of collagen in adult articular cartilage[73]. Subsequent translation of the IIB mRNA isoform results in the formation of the procollagen α-chains (Fig. 2a). Three procollagen α-chains coil around each other to generate a procollagen right-handed triple helix (Fig. 2a). Centrally located glycine residues allow tight packing of the helix[75]. The N-terminal and C-terminal are known as pro-peptides and do not assemble into the triple helix but are crucial for extracellular polypeptide secretion and helix formation, as disulphide bonds form between these regions[76]. Procollagen is then released into the ECM and the pro-peptides are cleaved by proteinases before being deposited together with collagen XI into the collagen fibrils[76] (Fig. 2a). Pro-peptide removal leads to reduced solubility of the procollagen, which then assembles into a highly organized tropocollagen structure. Finally, adjacent tropocollagen molecules assemble in a highly intricate pattern to build fibrils and fibres that have a large diameter[76] (Fig. 2a).

Fibrillogenesis and self-assembly

Fibrillogenesis is a step-by-step process that begins when chondrocytes release collagen molecules. These individual collagen molecules (~300 nm long and 1.5 nm in diameter)[77] fuse together laterally or linearly, forming large fibrils with the collagen triple helices aligned in the same direction within the fibril[78]. Different populations of thin (~16-20 nm in diameter) and thick (~40 nm in diameter) fibrils are described in the articular ECM[78]. Collagen type XI is predominantly found in the thinner fibrils, acting as a fibrillogenesis nucleator. Two collagen type II and two collagen type XI microfibrils form the fibril core, surrounded by ten collagen type II microfibrils (known as the '10 + 4' arrangement)[7] (Fig. 2b). Initially, smaller fibrils are formed, which interconnect over time to form larger fibril bundles (also referred to as fibres, composed of multiple fibrils). Collagen type XI can also crosslink with other collagen type XI molecules[79]. Other types of collagens, such as type IX, are present in smaller amounts and help to stabilize the heterotypic collagen II–XI fibrils[76]. Collagen type IX is a fibril-associated collagen with interrupted triple helices. This collagen forms covalent bonds with collagen type II and other type IX molecules and also crosslinks collagen type II with other ECM components[80,81]. Collagen type IX is located on the surface of thin fibrils in adult cartilage, disappearing from the thicker fibres as cartilage matures[8]. Its N-terminal non-collagenous domain 4 projects away from the collagen fibrils[82], interacting with ECM components, such as biglycan[80] or fibronectin[81]. Collagen type II, IX and XI ratios can vary, with an 8:1:1 ratio present in thin fibrils during development[83].

Collagen fibre diameter increases with growth and cartilage depth[84] (Fig. 2c). Fibril synthesis is influenced by various mechanisms, including growth inhibition, degradation and interactions with small proteoglycans that regulate fibril formation. Additionally, limited fibril diffusion in proteoglycan-rich areas can further slow fibril growth[85]. Thicker fibres, which lack collagen IX and XI, often contain decorin, a small proteoglycan that binds non-covalently and maintains proper separation between fibres – especially in deep and superficial regions where fibres run parallel to each other[8]. Decorin also enhances adhesion between aggrecan molecules and between aggrecan and collagen[8,86]. Therefore, collagen fibrillogenesis takes place on a densely populated matrix, where small proteoglycans and other ECM components create a natural molecular crowding effect, that catalyses fibrillogenesis[87]. This molecular crowding effect, and the high affinity of collagen II for molecules that regulate fibrillogenesis, contribute to fibril formation[85].

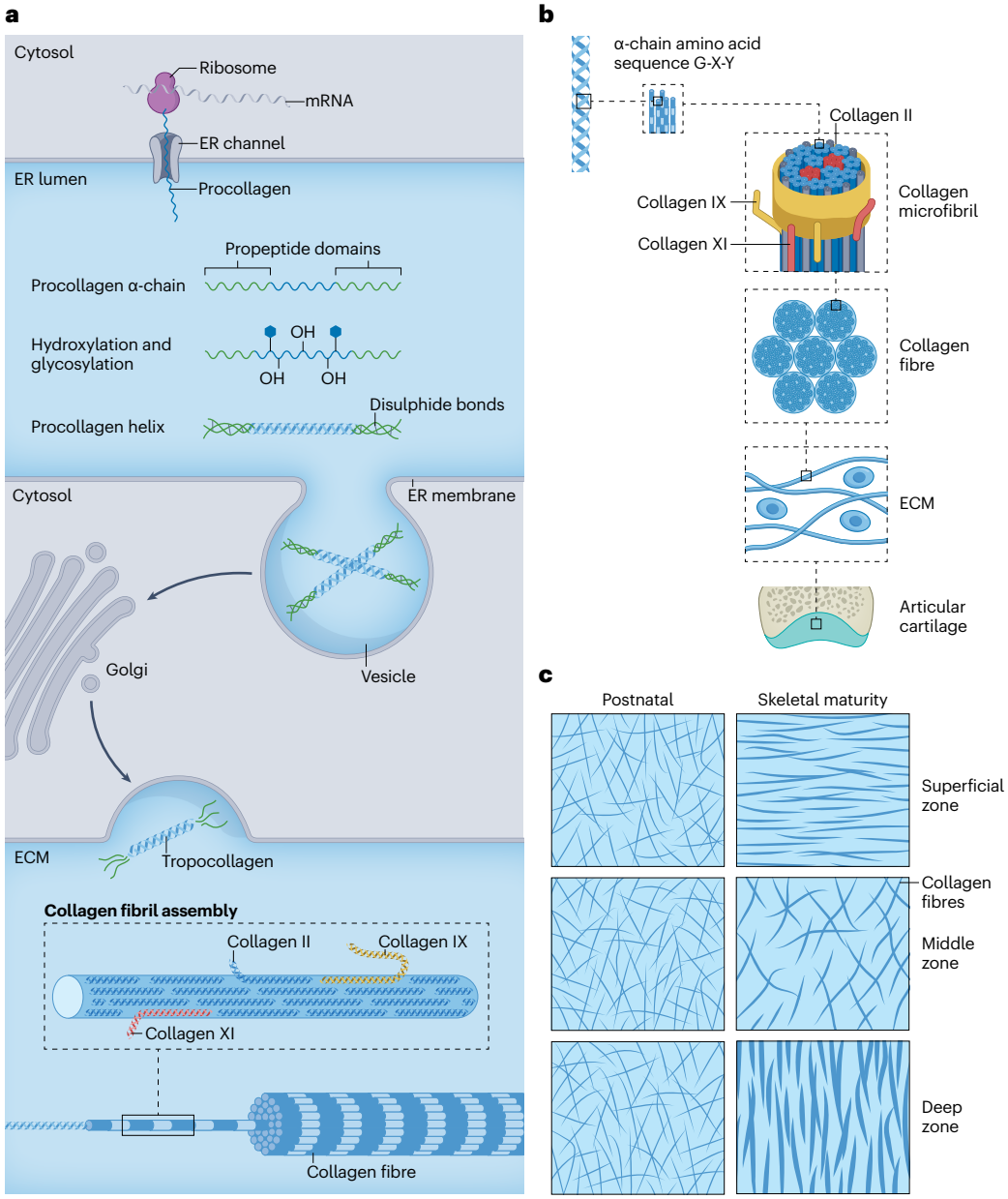

Fig. 2 | Collagen fibrillogenesis. a, Procollagen is released into the endoplasmic reticulum (ER). Procollagen α-chains have tripeptide glycine-X-Y (G-X-Y) repeats that are common to all collagens and are essential for the assembly of the α-chains into a procollagen triple helix (the X and Y residues are typically proline and hydroxyproline respectively[76]). Prior to forming the procollagen triple helix, these chains undergo post-translational modifications in the lumen of the ER[247] (hydroxylation and glycosylation). The procollagen helix is then transported to the Golgi, where it is processed further and finally transported in a vesicle into the extracellular matrix (ECM)[75]. Propeptides (green) are cleaved to form tropocollagen molecules, that then self-assemble into fibrils, incorporating as collagen type XI (red) and fibril-associated collagens (such as collagen type IX (yellow))[78]. **b**, The collagen fibril core is formed by two collagen type II and two collagen type XI microfibrils, which are surrounded by ten additional collagen type II microfibrils. Multiple collagen fibrils organize into larger structures, known as collagen fibres. **c**, During cartilage maturation, the number of crosslinks within collagen fibres increases, which leads to the formation of thicker and more mechanically robust fibres[89]. Collagen fibres also undergo reorganization; in the superficial zone, fibres align parallel to the surface, whereas in the deep zone, they adopt a vertical orientation[9]. mRNA, messenger RNA; OH, hydroxyl group.

Cartilage collagen is further interconnected by crosslinking, which is mostly facilitated by the enzyme lysyl-oxidase (LOX)[88]. As cartilage matures, both the nature and number of crosslinks increase, contributing to the increased stiffness and brittleness that is associated with aging[89]. Initially, the collagen fibrils contain reducible crosslinks (specifically, divalent ketoimines[90]), which gradually convert into non-reducible crosslinks (specifically, trivalent pyridinolines). Besides the regulation of fibre formation and crosslinking, fibre degradation

is also essential for shaping the collagen network. Chondrocytes produce matrix metalloproteinases (MMPs) that specifically target and degrade collagen, destabilizing fibrils by targeting regions involved in crosslinking. Additionally, other mechanisms, such as the oxidative state of the ECM or advanced glycation end-products, influence the balance of crosslinking in mature collagen fibres[89]. As mature articular cartilage collagen is slowly renewed and is deposited mainly during growth[17], its crosslinking is crucial for joint longevity and resistance to mechanical failure. The identification of the factors that drive initial crosslink formation and those that regulate crosslinking could elucidate why joints fail earlier in some individuals than in others. Altogether, larger-diameter fibres, collagen structure and collagen–aggrecan and collagen–collagen crosslinking further enhance ECM stiffness, leading to increased resistance to compression with maturation, especially in the deeper zones. These insights could inform future tissue engineering approaches.

Postnatal collagen structure reorganization

Considering the composition, structure, postnatal development and poroelastic behaviour of articular cartilage, it is evident that collagen structure and architecture have a fundamental role in shaping tissue mechanics. Collagen production, fibrillogenesis and tissue growth are crucial for this architecture; however, the exact mechanisms underlying the formation of the collagen arcade-like structure remain elusive. Current research efforts face two major challenges[91]: first, it is difficult to directly visualize collagen network formation and, second, the pace of collagen synthesis is relatively slow. Several hypotheses have emerged to explain how the structure of articular collagen develops. Data from studies on collagen reorganization in vertebrates (such as sheep[92], pigs[84], horses[93], rabbits[94] and opossums[95,96]) suggest that there are similarities in the remodelling processes among different joints and different terrestrial species. However, the articular cartilage collagen structure found in aquatic mammals is strikingly different, with a more random orientation, probably owing to the loss of terrestrial loading-based evolutionary pressure[49]. Therefore, articular cartilage development enables the tissue to adapt to increased loading[97] through the reorganization of the collagen type II fibres in the late prenatal and early juvenile stages up until skeletal maturity[94]. Notably, collagen molecules incorporated into fibres during development persist throughout life[17]. Termination of growth activity in the epiphyseal bone usually coincides with the maturation of the articular cartilage structure and the development of an anisotropic collagen organization[94]. This section describes the possible mechanisms that lead to collagen reorganization and highlights the need for further research to fully elucidate the orchestration of this complex process.

Collagen network formation in the deep zone

For fibrous tissues, such as tendons, fibroblasts align with the principal stress direction, producing collagen type I fibres that extend into the matrix in the same orientation. This alignment occurs within specialized extra-cellular cell membrane invaginations, known as fibripositors, in which fibres self-assemble after collagen monomers are secreted by fibroblasts[98]. Unlike collagen type I, active guidance of collagen type II by chondrocytes has not been demonstrated. In fact, the orientation of collagen type II fibres is considered a cell-independent process[99]. Thus, given that the chondrocytes in articular cartilage do not polarize, are encapsulated in a PCM and are sparsely distributed within the articular ECM, these cells are unable to directly reorganize collagen fibres or guide their orientation. Nevertheless, collagen alignment is probably indirectly related to tissue growth during postnatal development. Notably, collagen type II fibres are anchored to the subchondral bone[69]. During postnatal development, chondrocyte progenitors give rise to daughter cells that synthesize new ECM, which drives articular cartilage growth from the bottom up[94]. During this process, collagen fibres that are anchored to the subchondral bone could be pulled upwards with perpendicular-to-surface tissue growth, and new collagen monomers integrated into the fibres in this direction[100]. This mechanism has been demonstrated in vitro by stimulating neo-cartilage bottom–up tissue growth in a Transwell system[100,101]. Therefore, postnatal cartilage growth could be responsible for the arrangement of fibres in the deep zone, not directly by cell deposition, but by indirect effects of tissue growth (Fig. 3), which would explain the perpendicular orientation of fibres from the subchondral bone towards the surface in the deeper zones of the articular cartilage[100].

Collagen network formation in the superficial zone

Tightly packed, tangentially oriented collagen fibres provide crucial support for shear resistance and normal mechanical function of the articular cartilage[102]. In some species (mostly those that require locomotion immediately after birth[103]), the mechanical forces that occur from initial movements are an essential factor in joint formation[104–106], and lack of intrauterine fetal or postnatal joint motion (which can be caused by conditions such as developmental dysplasia of the hip or congenital hip dislocation[107]) results in abnormally shaped[108–110] or underdeveloped joints[107,111]. Several theoretical models that predict the structure of collagen networks across different tissues are built on the principle that collagen orientates in the direction that maximizes its function[112] (such as resistance to the positive principal strain[113,114]). In articular cartilage, this principle is in line with in vivo[115,116] and in vitro[117] studies that suggest that increased loading during late prenatal and postnatal development could be responsible for the parallel collagen fibre alignment on the cartilage surface (Fig. 3). Research on the postnatal development of calf femoral head cartilage demonstrated the presence of split lines predominantly in regions subject to major load-bearing stress[116]. These split-line patterns, which indicate the predominant tangential collagen orientation, only appear during postnatal development within the superficial zone, and are absent in newborn cartilage, suggesting progression linked to mechanical stimuli[118]. Another study highlighted that porcine knee cartilage from meniscus-covered regions, which experiences lower mechanical loads, has less parallel collagen alignment in the superficial zone, with more randomly orientated fibres and a lower density, compared with regions that are not covered by the menisci[115].

Overall, newly synthesized collagen fibrils are dispersed in the surrounding ECM. After collagen release, the loose fibrils are assembled in an isotropic manner into the existent network[72]. Therefore, when the superficial area experiences increased loading after birth, the collagen fibres begin to arrange themselves in a parallel pattern, probably owing to external loading forces[91,100]. This hypothesis is consistent with research that shows that the surface collagen fibre reorganization coincides with increased postnatal joint loading[115,119–122]. In addition, as the ECM in the deep zone expands, the superficial zone becomes compressed, which results from the combined effect of joint loading and bottom–up growth of deeper tissues[100]. Mechanical loading might also facilitate this compression by causing the release of water in the surface zone rather than in the deeper zones, in which the proteoglycan content and therefore osmotic potential are higher[28,123]. Also, the articular surface grows and stretches during development, creating

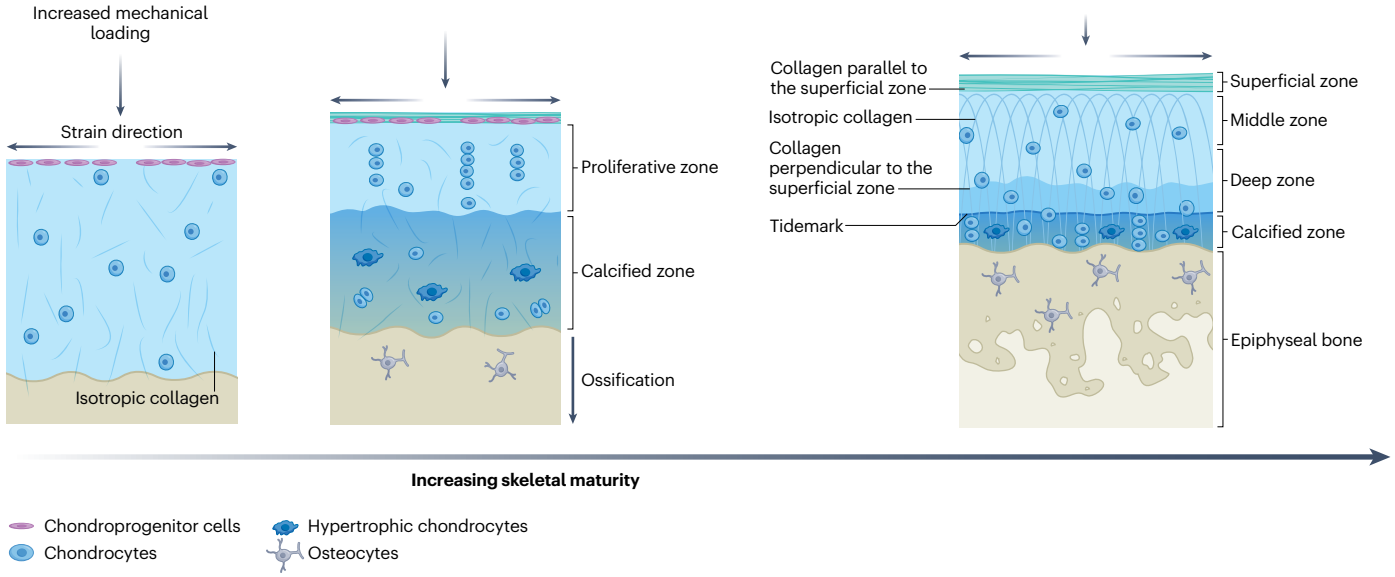

Fig. 3 | Proposed mechanism for the guidance of collagen type II fibres in articular cartilage. During postnatal development, bottom–up tissue growth is not driven by internal tissue remodelling but rather by tissue neoformation that occurs as a result of columnar division of chondrocytes in the proliferative zone, leading to bottom–up tissue growth that pulls collagen fibres into a perpendicular alignment relative to the cartilage surface in the deep zone[100,101]. Additionally, increasing mechanical load promotes the alignment of collagen fibres parallel to the surface in the superficial zone[115,119–122]. The articular surface also grows and stretches during development, creating tangential strains that pull the collagen fibres parallel to the surface[100]. Meanwhile, articular cartilage functions as a surface growth plate for the underlying epiphyseal bone; chondrocytes undergo hypertrophy, creating calcified bone tissue.

tangential strains that could potentially pull the collagen fibres parallel to its surface[100] (Fig. 3).

Taken together, these observations point towards a biomechanically driven hypothesis of collagen realignment, in which the orientation of collagen fibres is influenced by the forces generated within the ECM in response to tissue growth (which guides collagen alignment in the deep zone) and physiological loads (which guides collagen alignment in the superficial zone)[100]. Further understanding of this mechanically driven process could help to recreate collagen structures and achieve durable functional regeneration of the articular cartilage.

Proteoglycans in the articular cartilage matrix

Alongside fibrillogenesis, self-assembly and postnatal reorganization of collagen type II, the collagen network interacts with various other ECM components, which determines the overall mechanical properties of articular cartilage. The interplay between proteoglycans and collagen type II gives articular cartilage its unique poroelastic behaviour[124]. The water-attracting properties of the GAGs are constrained by the arcade-like collagen fibre network, building up a swelling pressure and enabling the tissue to withstand repeated mechanical loads over a lifetime. In the absence of external forces, collagen tension balances the swelling pressure created by negatively charged GAGs. This internal tension places the collagen network under stress, even when unloaded. Under compression, the pre-stressed collagen fibres, especially in the deep zone, in which fibres are thicker, highly ordered, and aligned with the load direction, provide resilience, unlike the more disorganized fibres in immature cartilage[125]. As proteoglycan density decreases towards the superficial zone, the collagen network experiences less swelling pressure, leading to lower fibre pre-strain in the middle zone[126,127]. Under physiological articular loading rates, the pressurization of the fluid retained by the GAGs mainly supports the load[10]. In addition to generating swelling pressure, the collagen network restricts proteoglycan movement, thereby resisting the outflow of interstitial fluid from the articular cartilage (which has low permeability)[91]. Aggrecan is the most abundant proteoglycan in articular cartilage and has a crucial role in load bearing. Its interaction with the collagen type II network is mediated through various molecular forces, including van der Waals, hydrophobic and hydrogen bonds and electrostatic interactions between the GAGs and positively charged amino acids on the collagen fibres[128]. Although aggrecan is the dominant proteoglycan found in articular cartilage, a range of smaller proteoglycans and glycoproteins also have an essential role in regulating collagen fibre organization and tissue homeostasis[70,129]. These small proteoglycans, such as biglycan, also contribute to more complex bridging interactions between aggrecan molecules and collagen fibres[86].

Aggrecan biosynthesis and deposition

Aggrecan is composed of a core protein that is covalently bound to sulphated GAGs, mainly chondroitin sulphate and keratan sulphate. The core protein of the aggrecan monomer is transcribed from the *ACAN* gene. The aggrecan core protein has five domains[130,131]: three globular domains (G1 and G2 at the N-terminal, and G3 at the C-terminal), an interglobular domain and an extended central domain with serine–glycine repeats (Fig. 4), in which the chondroitin and keratan sulphate chains bind[132]. Glycosylation occurs in the Golgi, where the first sugar of the GAG chain is added[133]. Various glycosyltransferases elongate the keratan sulphate and chondroitin sulphate chains[134] (Fig. 4). In humans, each aggrecan molecule contains 30–60 keratan sulphate chains and up to 100 chondroitin sulphate chains. After synthesis, proteoglycans are secreted into the extracellular space via secretory vesicles. Aggrecan

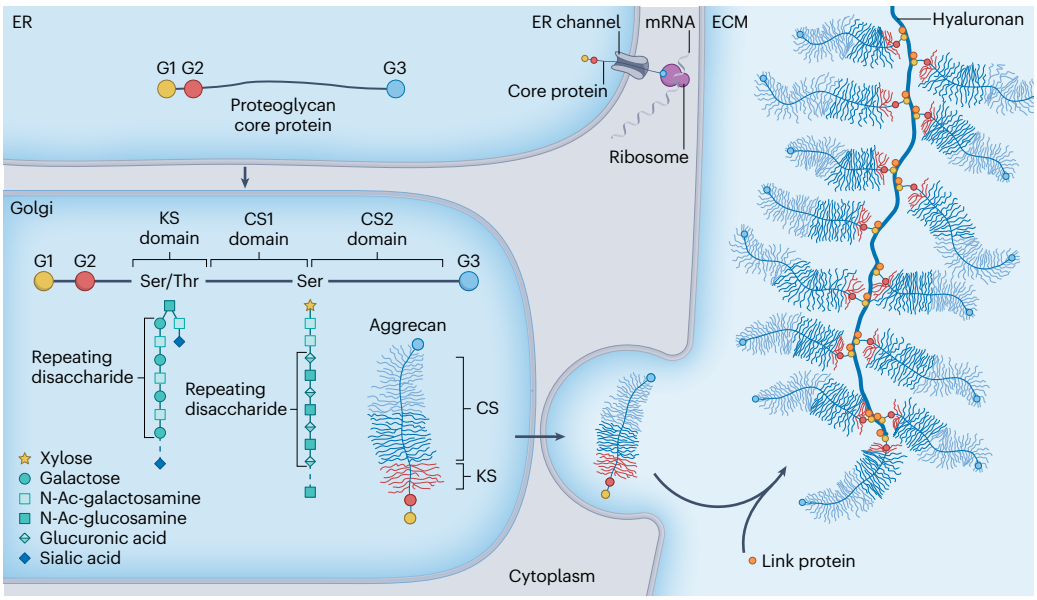

Fig. 4 | Proteoglycan aggrecan synthesis. The aggrecan core protein contains three globular domains (G1, G2 and G3) and a non-globular region between G2 and G3 for glycosaminoglycan (GAG) attachment. The aggrecan core protein is released into the endoplasmic reticulum (ER). Glycosylation occurs in the Golgi, where specific sugars are sequentially added to different non-globular domains to form the negatively charged GAGs keratan sulphate (KS) and chondroitin sulphate (CS)[134]. Following glycosylation, aggrecan is secreted in vesicles into the extracellular matrix (ECM). In the ECM, aggrecan binds to hyaluronan through the link protein, forming bottle-brush-shaped proteoglycan aggregates[135]. mRNA, messenger RNA; Ser/Thr, serine/threonine.

is found in aggregates in the articular ECM; hence, the name of this proteoglycan. It binds non-covalently around a central hyaluronan filament through the N-terminal G1 domain. This interaction between aggrecan and hyaluronan is stabilized by the presence of the link protein[135] (Fig. 4). Hyaluronan is formed in the cytosol and directly released to the extracellular space, forming a layer around chondrocytes[136].

Aggrecan structure and distribution

As previously mentioned, collagen fibres in the articular cartilage exhibit an anisotropic structure, and aggrecan has a depth-dependent gradient. At the surface, the proteoglycan content is relatively low, but it increases with depth[5], which raises swelling pressure and water content in the middle and deep layers. In the calcified cartilage, proteoglycan content decreases again, although aggregates are larger and more saturated with aggrecan[136]. These gradients influence tissue permeability, as negatively charged GAGs retain water, which impedes fluid flow; the availability of soluble growth factors and cytokines also varies with depth. The differential distribution of aggrecan is partially driven by the metabolic profile of chondrocytes and their depth-specific proteoglycan synthesis; the response of chondrocytes to mechanical loading varies across tissue zones[137]. Surface chondrocytes experience mostly shear and tensile forces, whereas deep-zone chondrocytes endure compression. In vitro evidence suggests that such distinct loading regimes promote specialized chondrocyte behaviour[138]. Moreover, aggrecan from the postnatal matrix has fewer fragmented monomers than adult cartilage, even in healthy adults, suggesting that this characteristic might be part of a natural homeostatic process[139] (Supplementary Fig. 1). Aggrecan fragmentation, even if physiological, becomes pronounced during OA, indicating its role in disease progression[140]. In addition to the fragmentation of the core protein, the mean size of the GAG chains also decreases with aging and in OA[141].

Other proteoglycans and glycoproteins in cartilage

The interaction between collagen type II and large proteoglycans, such as aggrecan, has a crucial role in tissue stiffness and resistance to deformation. This interaction is, in many cases, indirect and mediated through protein complexes consisting of small proteoglycans and glycoproteins. Small proteoglycans such as versican, biglycan and fibromodulin are essential for maintaining tissue integrity[142]. They closely interact with the arcade-like collagen network and perifibrillar adapter proteins, such as matrilins, regulating collagen-fibre diameter and ensuring cohesion within the ECM[85,143] (Fig. 5). As summarized in Table 1, these proteoglycans, glycoproteins and small proteins are essential for collagen fibrillogenesis, mechanical stabilization and ECM crosslinking.

Attempts to restore cartilage mechanical function

Once skeletal maturity occurs, the collagen matrix cannot be further replaced[144]. This lack of remodelling means that any damage to the arcade-like structure of collagen is essentially permanent[25]. Besides the limited regenerative capacity of articular cartilage, the absence of collagen matrix replacement and the lack of a collagen architecture accounts for the consistent failure to durably regenerate articular cartilage. Traditional approaches, such as autograft[19] and allograft[145] transplantation, have been used as a biological substitute for the damaged osteochondral tissue (Fig. 6a). Although these approaches include a replacement for the arcade-like network, they do come with considerable restraints. Besides potential limitations in availability, autologous transplantation can lead to tissue morbidity and dysfunction at the donor site[146], whereas allogeneic transplantation can trigger an immune response[147]. Moreover, structural integration of the grafts into the host tissue[148] can be a challenge and treatments will often fail in the long term. In view of the aging population, there is

therefore an urgent need for more durable approaches to articular cartilage restoration.

Novel clinical approaches, such as cell-based therapies, which involve the use of stem cells or chondrocytes (including expanded chondrocytes)[149], are combined with soft hydrogel-based scaffolds to keep the cells in place and provide infill for irregularly shaped defects to promote neocartilage tissue formation[150] (Fig. 6). Hydrogel materials, whether naturally derived (such as collagen, chitosan, silk fibroin, alginate, gelatine and hyaluronan)[151] or synthetic (such as polylactic acid and polyethylene glycol)[151], have been used to support chondrogenic differentiation. In addition, advanced composite hydrogels offer a more biomimetic approach than traditional injectable hydrogels by incorporating more than one synthetic material and/or key natural components of the ECM[152]; for example, blending collagen hydrogels with GAGs has led to hydrogels that can self-crosslink, which allows enhanced chondrogenic differentiation[153,154]. Also, the function of synthetic polymers can be improved with naturally derived ECM components[155]. Further research is needed to understand how additional components, such as other collagen types and small proteoglycans, could be incorporated. These other components have essential roles in fibril formation and stability and could further enhance the biomimicry of injectable hydrogels.

All of these therapeutic strategies for the restoration of articular cartilage incorrectly assume that the collagen network in mature individuals retains the capacity for remodelling[25]. Those developing therapeutic interventions should consider that the collagen network can barely adapt or rebuild in response to injury[144], and that the collagen synthesis rate by mature chondrocytes is low. With the emergence of rapid prototyping techniques, such as 3D bioprinting, the ability to precisely deposit cell-containing hydrogels (also referred to as bioinks[156,157]) has improved substantially[158]. Moreover, these technologies have enabled the creation of more complex structures that further resemble the native anisotropic tissue composition, potentially enhancing the functionality of engineered articular cartilage-like constructs[35,159,160]. Extrusion-based bioprinting is the most widely used technique[161,162]; however, more advanced printing techniques that can yield higher resolution (such as aerosol jet printing[163], inkjet bioprinting[164] and volumetric bioprinting[165]) have also been proposed. Although bioprinting can replicate the depth-dependent chondrocyte density gradients in articular cartilage[163], it fails to reproduce the collagen type II architecture and proteoglycan organization[166], leading to tissue with inferior mechanical performance[21].

To overcome this challenge, hydrogel-based bioprinting has been combined with other biofabrication approaches, such as melt-electrowriting (MEW) (Fig. 6) to generate articular cartilage implants with improved mechanical performance[167]. MEW utilizes an electric field to 3D print micro-scale polymer fibres, typically using polycaprolactone (PCL), with high resolution. This technique provides flexibility in fibre diameter, deposition patterns and layer stacking[168], and was successfully combined with multiple biofabrication techniques, including extrusion-based bioprinting[169], inkjet bioprinting[170] and volumetric printing[171]. The PCL-fibrous network generated by MEW provides a mechanical substitute for the collagen network in the articular

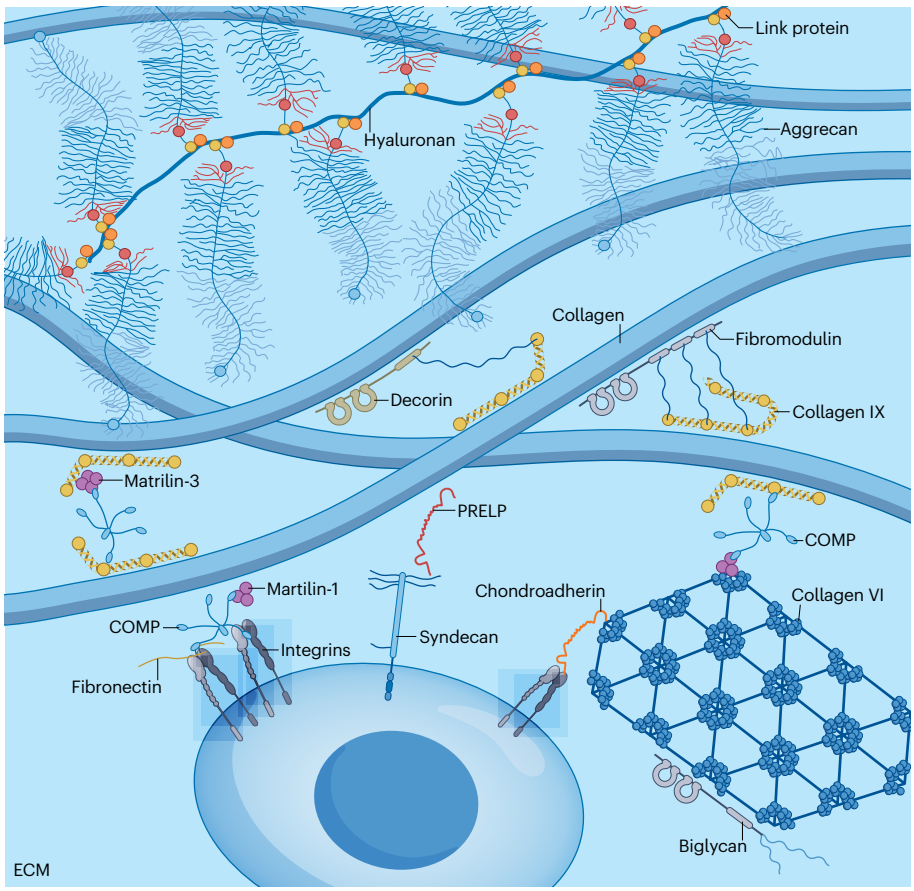

Fig. 5 | The main components of articular cartilage extracellular matrix. The extracellular matrix (ECM) of articular cartilage contains numerous components (only the main components are shown here, see Table 1 for a comprehensive list of ECM components). Heterotypic collagen fibres (blue) form a fibrillar collagen network. Aggrecan molecules bind to hyaluronan via the link protein, assembling into large bottle-brush aggregates that create a hydrated poroelastic matrix. Various interactions between collagen fibres and other minor collagens (such as collagen type VI and collagen type IX), proteoglycans (such as decorin, biglycan, fibromodulin, chondroadherin and PRELP), glycoproteins (such as martilin-1, matrilin-3, fibronectin and COMP) and chondrocytes (via cell-surface receptors, such as integrins and syndecans), ensure cohesion and structural integrity of the tissue. COMP, cartilage oligomeric matrix protein; PRELP, proline/arginine-rich end leucine-rich repeat protein.

Table 1 | The function of articular cartilage proteoglycans and (glyco)proteins and their interactions with extracellular matrix (ECM) components

Protein	Function	Interaction with ECM components	Refs.
Proteoglycans			
Versican	A large bottlebrush chondroitin sulphate-rich proteoglycan Has a role in mesenchymal condensation and chondrocyte differentiation during development via TGF-β signalling	Binds to collagen fibres and regulates their structure by increasing collagen condensation Interacts with hyaluronan, fibrillin, tenascin, fibronectin and heparan sulphate rich- proteoglycans to aid ECM crosslinking	217,218
Perlecan	A PCM heparan sulphate-rich proteoglycan Binds growth factors, such as FGF, to promote chondrogenesis Contributes to ECM mechanical stability	Interacts with collagen type VI and collagen type XI and other cell adhesive and structural glycoproteins to stabilize the chondrocyte PCM	219–221
Lubricin (PRG-4)	A mucinous glycoprotein on the articular cartilage surface Provides lubrication to the joint Protects articular cartilage from cell adhesion, inhibits synovial overgrowth and prevents cartilage–cartilage adhesion	Interacts with COMP, collagen type II and fibronectin in the superficial zone of the articular cartilage	222–225
Fibromodulin[a]	A keratan sulphate-rich proteoglycan Has an important role during development and mesenchymal condensation Surrounds cells at the articular cartilage surface during development and is becoming more widely detected across postnatal ECM	In adult articular cartilage, its levels correlate with the size of collagen fibres Influences fibrillogenesis and crosslinking by connecting collagen fibres with LOX	226,227
Lumican[a]	A keratan sulphate-rich proteoglycan Participates in host–pathogen interactions	Interacts with and restricts the growth of collagen type II fibres	228,229
Decorin[a]	A small proteoglycan with one GAG chain of chondroitin sulphate or dermatan sulphate Forms rings along the collagen fibres Its absence leads to a disorganized collagen network	Binds non-covalently to collagen fibres hindering the binding of other collagen molecules Regulates the lateral growth of collagen fibres, which controls fibre diameter	85
Biglycan[a]	A small proteoglycan with two GAG chains, either chondroitin sulphate or dermatan sulphate Has a role in collagen type II fibre formation Regulates morphogenesis and differentiation in the PMC	Regulates collagen fibre diameter Together with decorin, interacts with matrilin-1 to link collagen type VI (which organizes into beaded filaments that form hexagonal networks) to aggrecan and collagen type II	85,86
Chondroadherin[a]	A small cartilage matrix protein that mediates chondrocyte adhesion Has a role in cartilage homeostasis and morphogenesis Localized in the territorial matrix during development	Regulates cell–matrix interactions by binding collagen type II and collagen type VI to cell receptors (such as integrins and cell surface proteoglycans)	230,231
PRELP[a]	A proline and arginine-rich end leucine-rich proteoglycan Acts as a link between the ECM and cells in articular cartilage	Binds collagen and perlecan, acting as a link between the PCM and the territorial matrix	232
Other (glyco)proteins			
Matrilins	An adaptor protein for collagen and proteoglycans Pericellular matrilins allow chondrocyte mechanotransduction Has a role in the maintenance of chondrogenesis	In native articular cartilage these proteins bind mainly to collagen type IX on the fibre surface Both matrilin-1 and matrilin-3 regulate lateral collagen fibre growth	233
Thrombospondin	A matricellular protein Mediates cell–cell and cell–ECM interactions Has a role in articular cartilage formation and chondrogenesis	Regulates collagen fibre assembly by regulating collagen crosslinking enzymes, such as LOX	234
COMP	A pentameric glycoprotein, also known as thrombospondin-5 Involved in collagen organization Binds to ECM components, such as collagen, aggrecan, matrilins and fibronectin Involved in early limb development by binding to TGF-β and BMPs	Interacts and regulates collagen type II fibrillogenesis. Enhances fibre formation by bringing collagen together and catalysing fibrillogenesis with a uniform diameter	235,236
Fibrillin-1	A large ECM glycoprotein that forms a fibre meshwork within cartilage Together with elastin, contributes to the poroelasticity of the articular cartilage Its microfibrils regulate TGF-β bioavailability in the PCM (which in turn inhibits chondrocyte hypertrophy)	Involved in cell–ECM adhesion Interacts with perlecan and other molecules that attach to collagen	237–239

Table 1 (continued) | The function of articular cartilage proteoglycans and (glyco)proteins and their interactions with extracellular matrix (ECM) components

Protein	Function	Interaction with ECM components	Refs.
Other (glyco)proteins (continued)			
Elastin	A protein found in the ECM Provides tissue flexibility and maintains the elasticity of articular cartilage Can be involved in cell adhesion, migration and signalling	Bridging molecules anchor elastin fibres to collagen At the collagen surface, elastin fibres are oriented parallel to the collagen type II fibres; elastin is not detected in the deep zone	240,241
Tenascin-C	A glycoprotein involved in chondrogenesis during embryonic development Remains as an ECM component postnatally	Interacts with collagen type IX, acting as a link between chondrocytes and the ECM Connects chondrocytes and the surrounding ECM by binding to cell receptors	242
Fibronectin	A multidomain glycoprotein that forms a fibrillar network and requires the support of cell integrins for assembly Regulates ECM remodelling through interaction with α5β1 integrins	Binds to collagen type II and heparan sulphate proteoglycans, providing binding sites for cell adhesion proteins (such as syndecans and integrins)	243,244
Laminins	A large glycoprotein secreted by chondrocytes and located in the PCM Involved in chondrocyte adhesion, migration and resistance to apoptosis	Promotes chondrogenesis by increasing collagen type II, COMP and aggrecan production	245,246

[a]Indicates small leucine-rich proteoglycans. BMP, bone morphogenetic protein; COMP, cartilage oligomeric matrix protein; ECM, extracellular matrix; FGF, fibroblast growth factor; GAG, glycosaminoglycan; LOX, lysyl oxidase; PCM, pericellular matrix; PRELP, proline/arginine-rich end leucine-rich proteoglycan; TGF-β, transforming growth factor-beta.

cartilage and can remain stable in vivo, as demonstrated in horses, in which implants remained stable for 6 months[167]. However, this approach is only temporary as PCL is biodegradable and so will slowly disappear and restoration of the collagen network architecture is not induced[25]. Although this issue could potentially be addressed by the inclusion of non-degradable reinforcing polymer fibres (such as polypropylene[172]), true regeneration of the articular cartilage tissue can only be achieved by properly guiding the collagen type II network into its native architecture.

Attempts to incorporate collagen architecture

The anisotropic organization of the ECM, particularly the alignment of collagen fibres, is essential not only for the mechanical performance of cartilage tissue[173] but also for other tissues, such as the intervertebral disc[174], tendon[123], cornea[175], myocardium[176] and nerves[177]. However, current artificial scaffolds for treating articular cartilage damage cannot replicate the complex collagen type II architecture found in native tissue. Active guidance of collagen type II by chondrocytes has not yet been observed, unlike collagen type I, which is guided by fibripositors. Instead, as mentioned previously, the alignment of collagen type II fibres is thought to occur independently of cellular activity[99]. Therefore, if cells cannot directly guide collagen type II deposition, but collagen must be aligned in an arcade-like orientation to provide long-term support to articular cartilage implants, there is a need to address how to align collagen type II. By incorporating collagen fibrillogenesis cues (as discussed earlier in this Review) and orientation methods, it could be possible to improve collagen alignment within 3D-printed articular cartilage constructs, thereby enhancing graft mechanical stability[178]. Incorporating aligned and pre-aligned collagen structures has already shown promising results in promoting cell differentiation[179] and migration[180,181] across various tissues, offering a path towards creating a native-like ECM with long-term functionality and mechanical performance[25].

Approaches to inducing collagen alignment

For collagen type I, alignment can be achieved with fluid flow-induced shear forces, which induce collagen monomer orientation along the flow direction[182–186]. Alternatively, tension-based methods[187,188] (such as dip-pen nanolithography[189], which relies on fibre stretching), can be used to self-assemble the collagen monomers into networks similar to the natural fibres[190]. Given the challenges of mechanically orienting collagen, alternative approaches have been explored. These approaches include the use of thin aligned fibres obtained with electrospinning[191,192]. Collagen monomers are ejected from a nozzle under the influence of an electric field, leading to the formation of oriented fibres after solvent volatilization. Wet spinning offers a similar approach, whereby collagen polymer is dissolved in a suitable solvent and extruded through a spinneret into a solvent–non-solvent mixture[193]. Another non-mechanical method of collagen alignment is magnetic alignment[194]. Collagen has diamagnetic properties, which facilitates its perpendicular orientation to the direction of the magnetic field. Similarly, electrochemical alignment involves collagen assembly into fibres perpendicular to the electric field under an electrolysis solution[195]. Other techniques include collagen contact guidance, in which collagen adsorption capacity on the surface of specific substrates is used[180,196], or molecular crowding, whereby high-concentration collagen fibrillates into specific orientations under confined conditions[87,197]. Collagen strength depends not only on the alignment of the collagen fibres but also on their density, diameter and crosslinking. Research on fibrocartilage has demonstrated that the addition of exogenous LOX results in the alignment of and the formation of collagen fibres that are more mature[198]. Therefore, combining different bioactive stimuli for collagen alignment and fibrillogenesis is a potential strategy for developing functional collagenous tissues for repair and/or replacement in tissue engineering[199].

The application of these alignment techniques has not yet been explored for collagen type II fibres. In vivo, collagen type II fibres are thinner than collagen type I fibres, with less crystallinity and more binding sites for other collagen types and small proteoglycans[83,200,201]. This proteoglycan decoration limits fusion into larger fibres and instead promotes the formation of suprafibrillar assemblies through interconnected bundles. In vitro assembly and fibrillogenesis of collagen type II

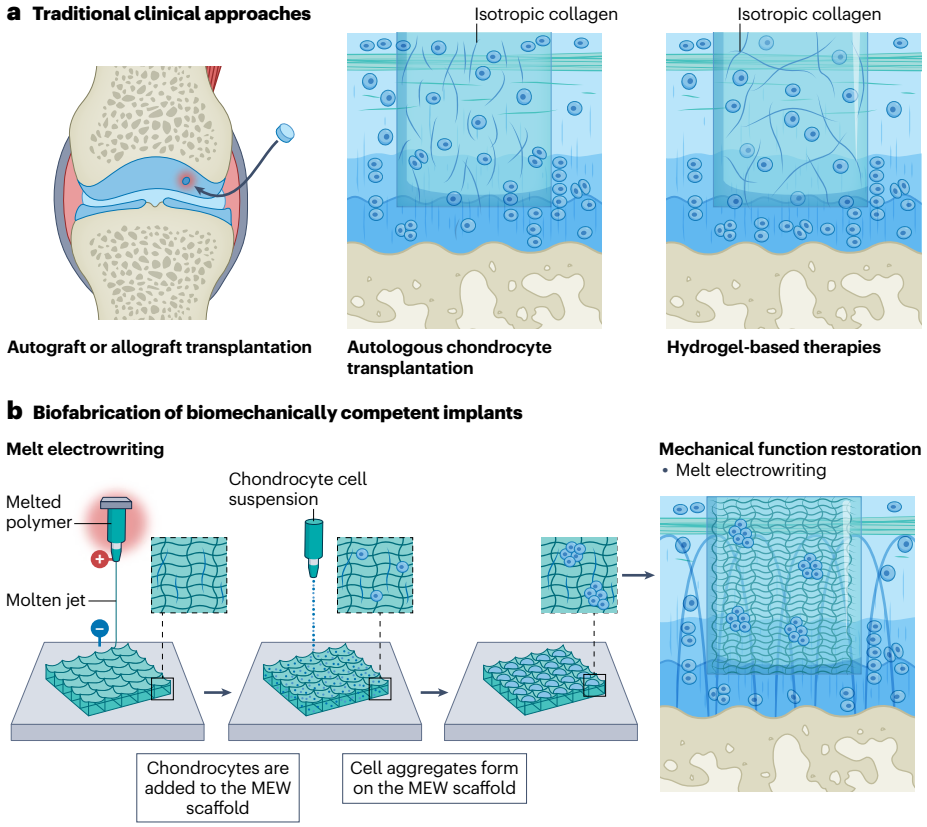

Fig. 6 | Strategies for guiding collagen in articular cartilage restoration and biofabrication. **a**, Traditional approaches to articular cartilage restoration include autograft[19] or allograft[145] transplantation, autologous chondrocyte injection[20] and the incorporation of chondrocytes into hydrogel-based scaffolds to enhance chondrogenesis and ECM production[150]. These hydrogels can be extrusion printed to create depth-dependent gradients[162]. **b**, Fibre-reinforcement technologies, such as melt electrowriting (MEW), enhance the mechanical properties of hydrogels, bringing the implants closer to mimicking native-tissue mechanics[168]. Moreover, spatial confinement of the fused spherical aggregates can give rise to an arched orientation of collagen in some regions[170]. **c**, Novel technologies for collagen alignment are aimed at developing implants with arched collagen networks and aligned collagen fibres, enhancing long-term mechanical properties. One approach involves mimicking tissue growth (top) to achieve vertical alignment of collagen type II in the deep zone by guiding ECM production through spatially directed TGF-β stimulation[100]. Other approaches focus on fabricating anisotropic hydrogels using novel technologies (such as magnetic microgel alignment (middle)[211] and filamented light biofabrication (bottom)[209]), creating aligned scaffolds that can guide collagen deposition in pre-cell-seeded implants. These approaches are aimed at producing implants with aligned collagen fibres and enhanced long-term mechanical properties.

is governed by liquid crystallinity properties at high concentrations, and the critical concentration for fibre assembly and nucleation rate are considerably different from collagen type I[201]. Moreover, although collagen type I fibre growth and orientation are achieved through active cellular processes[98], self-assembly is cell independent for collagen type II. Thus, fibre assembly is qualitatively different from collagen type I. Despite these differences most studies investigating the factors that influence collagen alignment in vitro focus on the orientation of collagen type I, and insights into the in vitro organization of collagen type II are lacking.

Mechanical loading could serve as a potential stimulus for aligning collagen type II, as it does during postnatal development, in laboratory-grown articular-cartilage scaffolds[202]. For instance, unconfined compression of chondrocyte-seeded agarose scaffolds leads to collagen fibre arrangement that is perpendicular to the direction of loading, therefore leading to fibre alignment parallel to the surface[117]. By contrast, radial confinement also promoted collagen-fibre orientation perpendicular to the scaffold surface[203]. Mimicking native compressive forces could offer a mechanical cue for achieving parallel and perpendicular collagen fibre orientation, representing the surface and deep zones of articular cartilage[202]. This approach was tested by applying mechanical cues to articular-cartilage scaffolds using a bioreactor system[204], which resulted in depth-dependent deposition of ECM components such as GAGs, but did not produce a collagen anisotropic network[204]. In addition to compression and shear forces, other biomechanical stimuli, such as tension, have been explored. Tensile forces also contribute to articular-cartilage homeostasis, and several tensile loading regimens have been reported to induce tissue formation and alignment of collagen fibres with tension[205]. Thus, although mechanically directing collagen type II assembly and orientation remains a challenge, it is one of the most promising approaches. Another area of increasing research is utilizing tissue growth as a potential mechanism for collagen type II alignment; for example, placing tissue-engineered neocartilage (derived from young bovine chondrocyte aggregates) in the upper compartment of a Transwell system[100] and adding TGF-β to the lower compartment (to promote bottom–up ECM deposition) resulted in perpendicular collagen alignment relative to the surface, similar to the alignment observed in the deep articular-cartilage zone (Fig. 6). However, the observed fibre alignment was limited to certain areas[100].

Novel techniques for collagen guidance cartilage

Novel fabrication approaches have helped to advance the guidance of collagen type II networks in engineered cartilage implants. Fibrous organized scaffolds generated with MEW technology have been used not only as a direct mechanical reinforcement for hydrogel-based implants[206] but also as a guiding structure for developing collagenous tissues[207]. On the basis of this principle, inkjet bioprinting was combined with MEW to precisely deposit cells into MEW-printed PCL grids[170], which resulted in cell self-assembly into cartilage aggregates that fused together to form hyaline-like tissue[170] (Fig. 6). The collagen-network orientation in the final engineered tissue appeared parallel near the surface and curved towards the deep zone. It is probable that the spatial confinement of the fused spherical aggregates within the developing tissue forced the collagen networks along the boundaries of the MEW fibres (Fig. 6). Although this approach did give rise to the correct orientation of collagen in some regions, especially the outer regions of the initial aggregates, it still did not reflect the true complexity of the native mature articular cartilage. However, this approach provided important proof that guiding collagen towards its native structure is feasible and could be combined with other additive manufacturing approaches to generate spatially organized osteochondral implants[208].

Besides MEW, the introduction of the filamented light (FLight) biofabrication technique[209] further highlights the ability to align collagen type II networks in chondral implants[210]. FLight generates hydrogels with aligned microfilaments and microchannels. These microchannels can promote directional collagen deposition by the cells and results in engineered tissue with aligned collagen and greater

Glossary

Anisotropic
Having direction-dependent properties.

Arcade-like structure
A collagen fibre arrangement in articular cartilage whereby fibres curve from the surface to the deep zone, anchoring to the subchondral bone.

Beaded microfibrillar network
A structural arrangement of thin, bead-like fibrils connected in a network.

Fibrillogenesis
The process of collagen fibril formation.

Heterotypic fibres
Collagen fibres composed of more than one type of collagen molecule.

Hypertrophy
The process by which cells undergo significant enlargement owing to volumetric increase and distinct metabolic and molecular changes.

Isotropic
Having uniform properties in all directions.

Joint cavitation
The formation of the joint cavity during embryonic development, separating the joint into two articular surfaces.

Liquid crystallinity
A state of matter with properties between those of a liquid and a solid crystal, characterized by ordered molecular alignment.

Molecular crowding
A high concentration of macromolecules in a confined space that influences molecular interactions and assembly.

Nascent
Newly formed or immature.

Non-reducible crosslinks
A non-reversible chemical bond that permanently stabilizes collagen fibrils.

Nucleator
A molecule or structure that promotes the initiation of fibrillogenesis.

Procollagen
The precursor molecule of collagen that undergoes enzymatic processing to form tropocollagen.

Reducible crosslinks
A reversible chemical bond that stabilizes collagen fibrils.

Resistance to the positive principal strain
The ability of a material to withstand tensile forces along the direction of the highest principal strain.

Split-line orientation pattern
A pattern observed on the surface of cartilage that reflects the underlying collagen fibre alignment.

Suprafibrillar assemblies
Higher-order structures formed by the organization of fibrils into complex networks.

Tidemark
Boundary between the calcified and non-calcified zones of articular cartilage.

Tropocollagen
The basic triple-helical collagen molecule that assembles into fibrils.

mechanical properties (Fig. 6). Similarly, aligned rod-shaped microgels have been explored to generate aligned ECM[211]. By using microgels with magnetic properties, the angle of alignment could be controlled and oriented to reproduce the articular cartilage architecture, with the deep zone microgels oriented perpendicular to the surface and superficial zone microgels oriented parallel to the surface[211] (Fig. 6). Other potential approaches include the development of porous scaffolds with anisotropic pore architectures that are designed to direct collagen type II deposition and create biomimetic articular-cartilage grafts[212,213]. These novel techniques represent major progress towards the ability to control and guide collagen type II alignment in engineered articular-cartilage constructs and could potentially overcome the limitations of current approaches.

Challenges and considerations

This Review explores the basic biology of articular cartilage, from its cellular and ECM composition in adult tissue to postnatal development that gives rise to the complex tissue architecture. We emphasize the importance of the adult articular-cartilage anisotropy and zonation, with a particular focus on the fibrillar collagen network and the proteoglycan-rich matrix. Collagen type II is the most important structural component of the articular matrix, with organization that gradually changes during postnatal joint morphogenesis leading to a functional arcade-like arrangement[25]. This reorganization of the collagen network and the proteoglycan matrix has a key role in the mechanical properties and stability of the native tissue and provides stiffness and compression resilience as the tissue develops, which enables load distribution across the joint and reduces friction during joint movement. Given the poor regenerative capacity of articular cartilage, any damage to the collagen arcade-like structure is potentially irreversible[25]. Therefore, novel approaches, including those using biofabrication, are required to address the mechanical challenges encountered in the restoration of cartilage[214]. Combining these approaches with collagen alignment techniques could offer a promising therapeutic strategy — not only to enhance post-transplant mechanical stability but also to align collagen in a way that further mimics the native structure and supports long-term function, even after the reinforcement degrades.

Several knowledge gaps remain. Current literature focuses mainly on inducing chondrogenic differentiation for ECM production (both in cell and/or hydrogel-based therapies and in laboratory-grown implants) and often overlooks the long-term ECM architecture of the resulting tissue. Although this organization is crucial for mechanical properties, the driving factors behind the ECM reorganization during development remain poorly understood. By using polarized light microscopy, many studies describe the transition from an isotropic to an anisotropic collagen network in the adult cartilage[120–122]. Emerging hypotheses suggest that collagen organization during development arises from a combination of appositional tissue growth and mechanical loading that increases postnatally[100]. Applying this knowledge effectively in functional and translational therapies requires a deeper understanding of all the factors that influence this process[25]. For instance, although several mechanotransduction pathways have been identified in chondrocytes, their potential role in postnatal ECM reorganization remains unclear. Additionally, the contribution of other ECM components to this reorganization, such as aggrecan, small proteoglycans and/or remodelling enzymes (such as MMPs and LOX), could pave the way for new therapeutic approaches. Understanding and combining novel technologies with insights into the basic biology of bioactive stimuli that drive ECM organization, such as mechanical loading, bottom–up tissue growth, collagen fibrillogenesis and fibre crosslinking, could lead to the development of tissues with the collagen content, crosslinking level and organization that can recover native mechanical support.

Although this Review focused mainly on structural aspects, it is also important to consider the inflammatory environment that is often present at sites of cartilage defect, especially in OA and traumatic injuries[215]. Inflammatory cytokines and matrix-degrading enzymes can compromise the integrity of both the implanted tissue and any collagen structures within it[216]. Thus, modulating this inflammatory environment through anti-inflammatory treatments or immunomodulatory strategies is essential before transplanting any engineered tissues[215]. Thus, to improve the success and durability of regenerative cartilage restoration, the structural ECM properties of the implant, as well as the inflammatory environment of the host tissue, will need to be addressed.

Conclusion

As the field of tissue engineering advances, new approaches to chondral implants and collagen guidance will continue to emerge, enabling the development of more biomimetic constructs. By replicating the native collagen architecture and proteoglycan composition of the native articular cartilage, better long-term mechanical stability of implants could be achieved, paving the way for improved post-transplant durability and clinical translation. Insights into the key aspects of articular ECM composition and the structural changes that occur during development can inform tissue-engineering therapies. Furthermore, in vitro collagen alignment techniques have the potential to be incorporated into the design of laboratory-grown implants. Learning from cartilage native architecture could open up the possibility of creating mechanically competent and durable tissue constructs that maintain their integrity after transplantation, ultimately helping to reduce the burden of joint disease.

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Competing interests

The authors declare no competing interests.

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