Narrative Review

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Preclinical models of endometriosis and interstitial cystitis/bladder pain syndrome: an Innovative Medicines Initiative-PainCare initiative to improve their value for translational research in pelvic pain

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Abstract

Endometriosis (ENDO) and interstitial cystitis/bladder pain syndrome (IC/BPS) are chronic pain conditions for which better treatments are urgently needed. Development of new therapies with proven clinical benefit has been slow. We have conducted a review of existing preclinical in vivo models for ENDO and IC/BPS in rodents, discussed to what extent they replicate the phenotype and pain experience of patients, as well as their relevance for translational research. In 1009 publications detailing ENDO models, 41% used autologous, 26% syngeneic, 18% xenograft, and 11% allogeneic tissue in transplantation models. Intraperitoneal injection of endometrial tissue was the subcategory with the highest construct validity score for translational research. From 1055 IC/BPS publications, most interventions were bladder centric (85%), followed by complex mechanisms (8%) and stress-induced models (7%). Within these categories, the most frequently used models were instillation of irritants (92%), autoimmune (43%), and water avoidance stress (39%), respectively. Notably, although pelvic pain is a hallmark of both conditions and a key endpoint for development of novel therapies, only a small proportion of the studies (models of ENDO: 0.5%-12% and models of IC/BPS: 20%-44%) examined endpoints associated with pain. Moreover, only 2% and 3% of publications using models of ENDO and IC/BPS investigated nonevoked pain endpoints. This analysis highlights the wide variety of models used, limiting reproducibility and translation of results. We recommend refining models so that they better reflect clinical reality, sharing protocols, and using standardized endpoints to improve reproducibility. We are addressing this in our project Innovative Medicines Initiative-PainCare/Translational Research in Pelvic Pain.

Keywords: Preclinical research, Endometriosis, Interstitial cystitis/bladder pain syndrome, Translational research, Pelvic pain, In vivo, Animal model, Rodent

1. Introduction

Animal models play a crucial role in research, helping to improve disease understanding and laying the foundation to develop new drug therapies. A wide spectrum of animal models has been developed and proven to be a useful tool in translational research. Ideally, such models need to replicate the aetiology and symptoms of the disorder under investigation as closely as possible. However, achieving this ideal situation is no easy task and may be hampered by an incomplete understanding of the

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.painjournalonline.com).

PAIN 162 (2021) 2349–2365

http://dx.doi.org/10.1097/j.pain.0000000000002248

disorder targeted for evaluation. Depending on the primary goal, animal models might replicate a disease of interest to different degrees. On the one hand, some animal models focus on further understanding basic science by looking into specific aspects of the disease; in these cases, they may resemble the disease alone partially. On the other hand, when models are designed to be used for the development of new therapies, they need to replicate both aetiology, disease mechanisms (construct validity), and disease symptoms (face validity).

Endometriosis (ENDO) is a chronic, hormone-dependent, peripheral neuroinflammatory condition associated with devastating pelvic pain.[56] The defining hallmark of the disorder is the presence of tissue fragments (lesions), similar to the endometrium, outside the uterine cavity, rather than in the centre of the uterus (eutopic endometrium). Three major subtypes of ENDO have been defined based on lesion locations: superficial peritoneal, ovarian (endometrioma/cysts), and deep ENDO. There are no effective biomarkers for ENDO, and definitive diagnosis is based on imaging (deep, ovarian cyst) or laparoscopic visualisation of the pelvis. Endometriosis is estimated to affect 6% to 10% of women of reproductive age[44] and up to 50% of infertile women.[95] The natural history of the disease remains poorly understood although genetic, immunological, and hormonal factors are all believed to contribute to disease severity.[44] Notably, although research has identified changes in the central nervous system of patients with ENDO, investigators report a poor correlation between pain severity and the amount, location, and subtype of endometriotic lesions, with some affected patients even being asymptomatic.[32] Currently, there is no cure for ENDO, and it is difficult to treat, with most patients offered surgery or drugs that symptomatically and inadequately treat pain or suppress oestrogen action/production causing temporary chemical castration. There are high recurrence rates after surgery, with estimates that lesions return in 40% to 50% of patients within 5 years.[49] Medical treatments are highly variable in their efficacy and acceptability,[133] with many patients having unwanted side effects including suppression of fertility and "menopausal" symptoms. Surveys of patients and health care professionals have consistently cited the need for development of new, effective, and noncontraceptive drugs to successfully control chronic pain symptoms.[14,57]

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a debilitating condition of unknown aetiology and without effective treatment. Interstitial cystitis/bladder pain syndrome is a symptom-based diagnosis requiring exclusion of other diseases and may have several different causes. A global consensus definition does not exist.[88] However, 2 distinct subtypes of patients have been distinguished: those with and those without Hunner lesions, which are diagnosed on the basis of cystoscopy. Management depends on the patient's subtype but is always symptomatic.[84] Recent epidemiological surveys estimated the prevalence of IC/BPS at around 45/100,000 in women and 8/100,000 in men.[103] To establish a diagnosis of IC/BPS, patients have to present chronic bladder pain and urinary symptoms such as urgency and frequency.[87] In addition, IC/BPS can be associated with a broad spectrum of comorbidities. These variations combined with the lack of knowledge concerning pathophysiology may explain why existing treatments have low efficacy.

Chronic pelvic pain (CPP)/urogenital pain is defined as "pain symptoms perceived to originate from pelvic organs/structures typically lasting more than 6 months. It is often associated with negative cognitive, behavioral, sexual, and emotional consequences as well as with symptoms suggestive of lower urinary tract, sexual, bowel, pelvic floor, myofascial, or gynecological dysfunction."[5] Examples of conditions associated with CPP are irritable bowel syndrome, vulvodynia, prostatitis, ENDO, and IC/BPS; all of them with a high medical need for new, effective treatments.[5]

In ENDO and IC/BPS, particularly, it has been reported that these conditions can also occur simultaneously.[25] Furthermore, ENDO patients have approximately a 4 times higher risk of developing IC/BPS compared with non-ENDO controls.[141] In addition, among patients with suspected IC/BPS, when subjected to cystoscopy, patients comorbid with ENDO have a stronger correlation with typical IC/BPS findings such as Hunner lesions, and reduced functional bladder capacity, compared with patients without ENDO.[54] All in all, because each condition individually has a strong impact on pelvic pain,[41,61,123] the quality of life of patients with both conditions, or with CPP, is consequently markedly reduced.[17,117,121,128]

Encouraging reports of progress towards new therapies based on preclinical research are tempered by relatively low success rates in subsequent clinical trials. A major factor for this discrepancy may relate to reproducibility, reliability, and robustness of the animal models used and whether they adequately reflect the full spectrum of symptoms and disease pathophysiology in patients,[50] especially regarding pain.[16] In this review, we have addressed this issue by considering evidence of the endpoints that have been explored in preclinical models of 2 different pelvic pain disorders: ENDO and IC/BPS. Rigorous evaluation of these endpoints needs to be considered to improve translational research for these disorders. Reviews highlighting the latest advances using preclinical models for each of these 2 diseases have been performed and discussed previously.[11,119] It is not the intention of this review to replicate findings, but rather to analyse, quantify, and compare the methodologies applied in the development of preclinical models and the outcome measures reported for each model type, with a critical view on parameters that may be of relevance in the clinic. We believe that by rigorously analysing these data, we can contribute to the development of improved preclinical models for both ENDO and IC/BPS, one of the main goals in our Innovative Medicines Initiative (IMI)-PainCare project, subproject Translational Research in Pelvic Pain (TRiPP) (https://www.imi-paincare.eu/PROJECT/TRIPP/).

2. Methods

2.1. Literature search

Two independent literature searches were performed using the advanced search engine from the Embase database (https://www.embase.com/#advancedSearch/default). For the first search, the terms "endometriosis" and "model" were used. For the second search, the terms "interstitial cystitis" or "bladder pain syndrome" were used. The advanced search settings included the following mapping options: "map to preferred term in Emtree," "search also as free text in all fields," "explode using narrower Emtree terms," and "search as broadly as possible." The first 2 searches were limited to show results published in the last 20 years (between 1998 and May 2019). Subsequently, 2 new searches with the same search criteria were performed in June 2020 covering all publications that arose between May 2019 and June 2020.

Overall, searches for ENDO and IC/BPS led to 3309 and 7266 results, respectively, which were exported into RIS (research information system) file format and opened in the software EndNote X7 (Clarivate, Philadelphia, PA) for further analyses.

Data from the search results were further automatically and manually analysed for systematic article selection and subcategorisation of preclinical models (**Fig. 1**). A detailed description of article selection, definition of subcategories, and analysis of construct and face validity can be found in the supplementary data (available at http://links.lww.com/PAIN/B319).

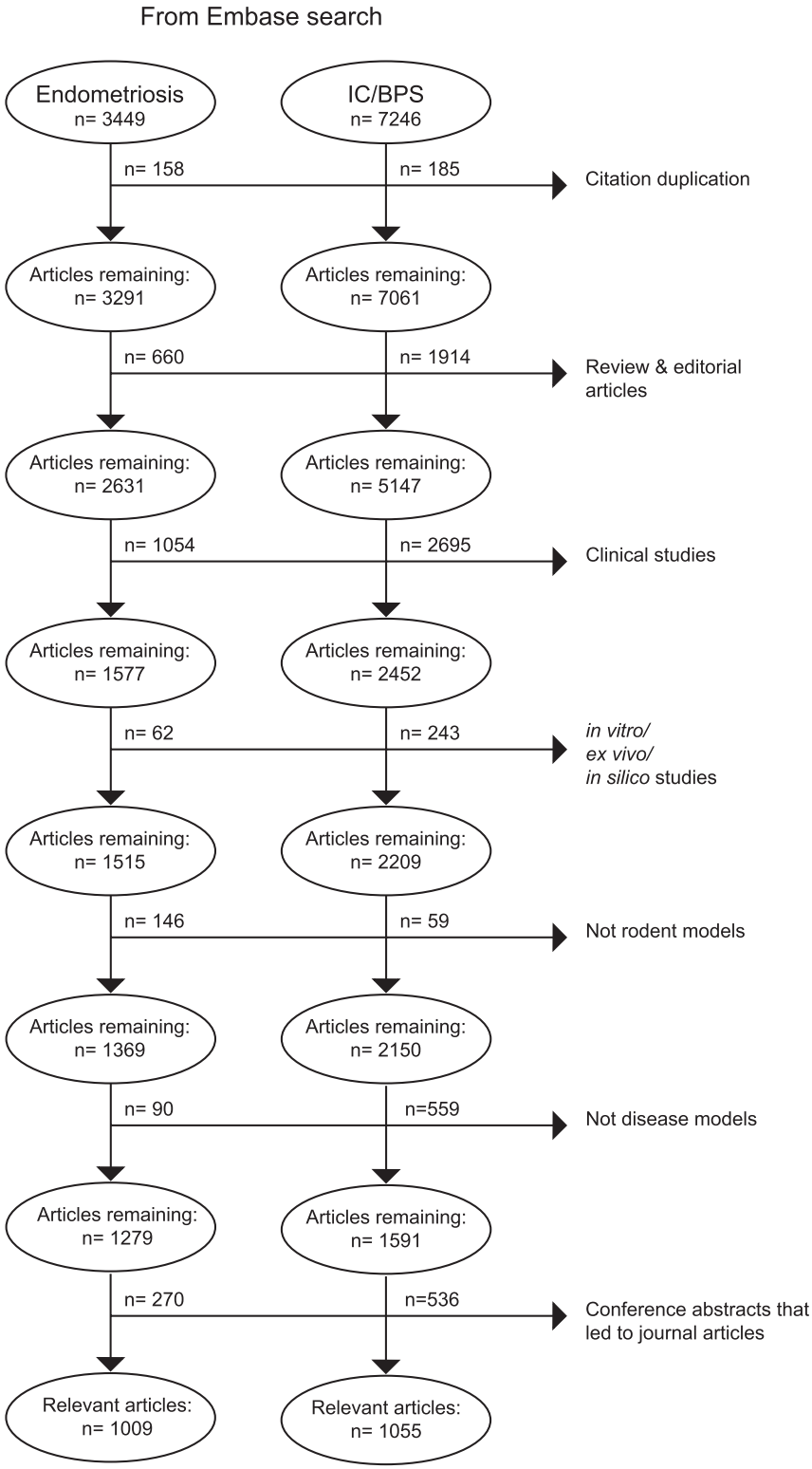

Figure 1. Selection of relevant research articles using endometriosis and IC/BPS preclinical models. Articles from the Embase database were imported into the software EndNote and classified. The order of classification was defined as shown in the flow chart: first, "reviews and editorials," then "clinical studies," then "in vitro/ex vivo/in silico studies," then "not rodent preclinical models," and finally "not endometriosis" or "not IC/BPS models." To avoid duplication of data, all poster presentations that had led to publications were excluded from the analysis, as well as poster presentations of the same data published more than once. Articles remaining were categorized as "ENDO preclinical rodent models" or "IC/BPS preclinical rodent models" and consequently analysed. ENDO, endometriosis; IC/BPS, interstitial cystitis/bladder pain syndrome.

3. Results

3.1. Preclinical models of endometriosis

During the last 2 decades, 4 different transplantation techniques have mainly been used: xenograft transplantation (transplantation of endometriotic tissue of human origin into immunocompromised mice), syngeneic transplantation (transplantation of uterine tissue of murine origin into recipient mice), autologous transplantation (transplantation of uterine tissue (uterine horn/uterus fragments) of rodent origin into the diverse pelvic regions of the same animal), and allogeneic transplantation (transplantation of uterine tissue from one mouse into the same mouse).

In our search, we found that from a total of 1009 scientific publications using preclinical models of ENDO (**Fig. 2A**) 41% used autologous transplantation, 26% syngeneic transplantation, 18% xenograft transplantation, and 11% used allogeneic transplantation. A minority of publications (3%) did not specify the model used, and very few used other means to induce ENDO, eg, CRISPR/Cas gene editing. We evaluated the number of articles being published per year for each category, and it was apparent that the most commonly used ENDO model was also the first widely established methodology in rodents: publications using autologous transplantation increased every year until 2015, reaching a peak of 43 publications (**Fig. 2B**). Since then, there has been a slight reduction and a plateau, likely reflecting the adoption of other models as detailed below. Syngeneic transplantation is the second most widely used method, and starting in 2008, there was a rapid increase in the number of publications using this model, peaking in 2019 with 41 publications. The xenograft transplantation model (first described in 1999) has not been as widely used, likely reflecting limitations in access to human tissue/cells and the requirement for immunosuppressed recipients. Although the number of publications increased between 2008 and 2015, reaching a peak of 28 publications, there was a decrease in the following years. The allogeneic transplantation model is the least used model variant. This was first described in 2000 and was increasingly reported from 2010, reaching a peak in 2016 with 13 publications. It is important to note that during the analysis of the search results it became clear that a contributing factor to the high number of publications within each category comes from laboratory groups publishing their discoveries using the same model repeatedly over time (not shown), and that this is also of concern when reproducibility of data becomes an issue.

Furthermore, differences exist between the model subcategories in the methods used to deliver endometriotic/uterine tissue and the location of the tissue implants (**Figs. 2C–J**). In the autologous transplantation model, suture is the only method used to develop "lesions" (**Figs. 2C and D**). Considering the location of implanted tissue fragments, 33% of publications reported suture to the "peritoneum," followed by the "abdominal wall" (26%). Since both refer to the same target tissue area, together they accounted for 59%, ie, the most targeted area in this model category. In addition, 19% of publications located tissue on mesenteric arteries/vessels, 3% on ovaries, and a minority (3% of total, 6 publications) in the colorectal region. Eight percent of publications sutured tissue fragments to other locations (eg, gastrocnemius muscle,[4] bowel mesothelium,[9] sciatic nerve,[23] and hypochondriac regions[20] or used dorsal skinfold chambers[72]). Ten percent of publications did not specify the location of implanted tissue fragments.

In the syngeneic transplantation model (**Figs. 2E and F**), the most common route of delivery was by intraperitoneal injection of homogenized tissue fragments (62% of all publications in this category), followed by suturing or gluing uterine fragments intraperitoneally (32% of publications), and, finally, a minority of publications reported subcutaneous injection of suspended tissue (less than 2% of publications[37]). Four percent of publications within this category did not specify either the route of delivery or the location of the implanted tissue.

In the xenograft transplantation model (**Fig. 2G and H**), the most common route of delivery was intraperitoneal injection of suspended endometriotic tissue (53% of all publications in this category), followed by suturing or gluing endometriotic tissue fragments (24% of publications) and, finally, subcutaneous injection of suspended tissue (15% of publications). Nine percent of publications within this category did not specify either the delivery route or location of the implanted tissue.

In the allogeneic transplantation model (**Figs. 2I and J**), suturing was the most common method used to secure uterine tissue implants. The "peritoneum" (53%) was the most commonly targeted area, followed by mesenteric arteries/vessels (21%), and the "abdominal wall" (15%). Together, the "peritoneum" and the "abdominal wall" represent 68% of publications of the model category. Eleven percent of all publications did not disclose where tissue fragments were implanted.

3.2. Construct validity for preclinical models of endometriosis for translational research

We developed an algorithm with a 3-point scale to determine the models and delivery methods better suited for translational research in terms of construct validity. We found that the construct validity ranking for ENDO models was as follows (**Fig. 3**): the highest rank was reached by the syngeneic model performed by intraperitoneal injection of suspended tissue, with a score of 12 points. The second rank, with a score of 8 points, is shared by the allogeneic model and the syngeneic model, both performed by surgery. The third rank, with 6 points, is shared by the xenograft model by intraperitoneal injection and the autologous transplantation model, which is performed by surgery. The fourth rank, with 4 score points, is shared by xenograft performed by surgical implantation and syngeneic transplantation by subcutaneous injection. Finally, in the last rank with 2 points is xenograft transplantation performed by subcutaneous injection of endometriotic tissue. Interestingly, the model subcategory with the highest ranked score in our construct validity scale, syngeneic transplantation by intraperitoneal injection, is also the second overall most used preclinical model of ENDO, with 167 publications, and the most used model per year since 2017.

3.3. Face validity in preclinical models of endometriosis

All in all, the lesion size was the most addressed endpoint parameter in model subcategories of ENDO, being analysed in 53% of publications using the autologous transplantation model, 74% from syngeneic, 77% from xenograft, and 80% from the allogeneic transplantation model (**Figs. 4A–D**). The second most reported endpoint was inflammation, being measured in 31% of publications using autologous, 40% using syngeneic, 19% using xenograft, and 47% using allogeneic transplantation models. Although adhesions occur in a high proportion of patients, a specific evaluation of adhesions in the preclinical models was rarely evaluated (12%, 8%, 5%, and 14% of publications from each model subcategory as defined above).

Notably, one of the key clinical symptoms reported by those with ENDO is CPP, and this has not been rigorously and reproducibly analysed in preclinical models of ENDO. As pain

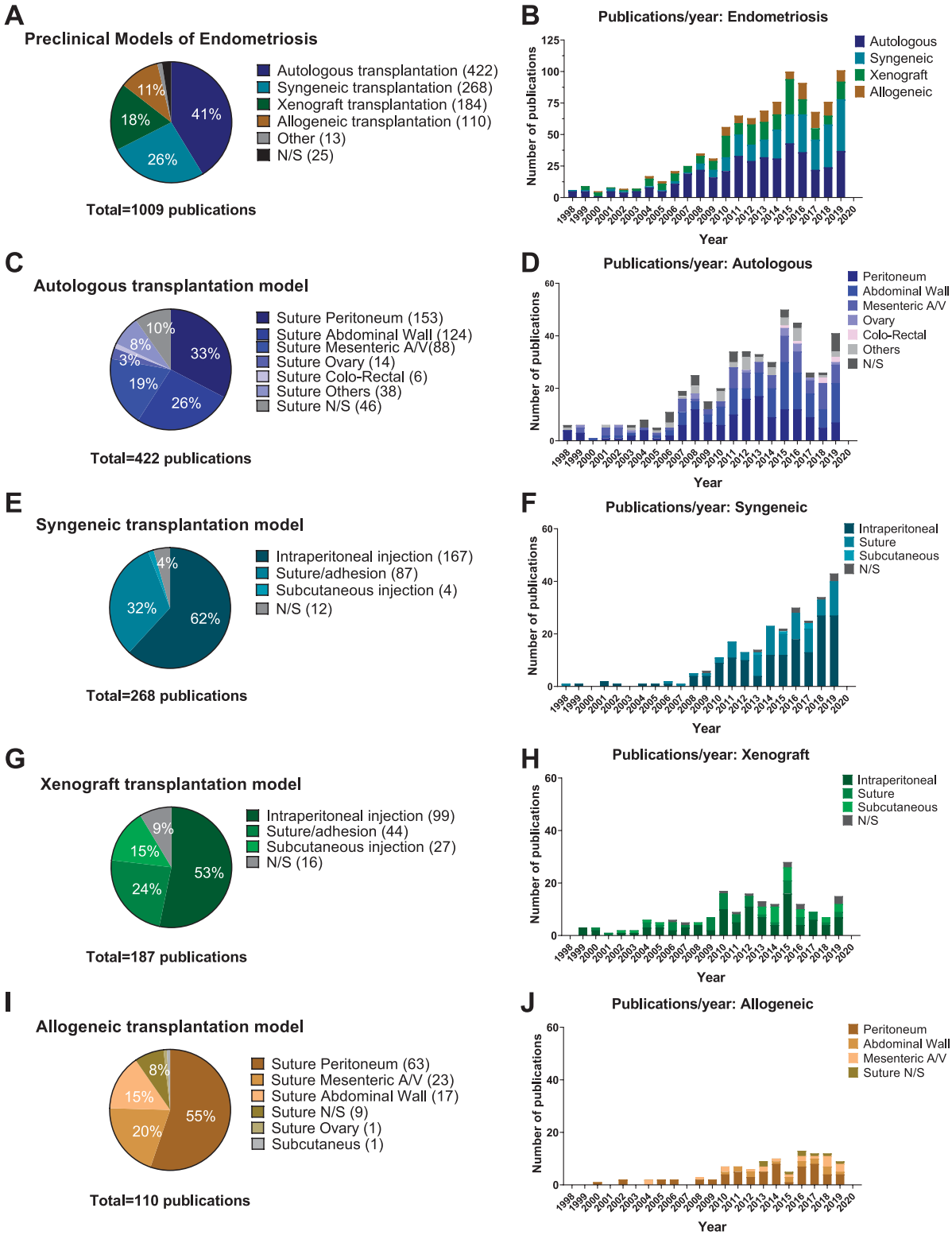

Figure 2. Preclinical models of endometriosis. (A) Four main categories of preclinical models of endometriosis were found. Blue fraction: autologous transplantation, turquoise fraction: syngeneic transplantation, green fraction: xenograft transplantation, and brown fraction: allogeneic transplantation. (B) Number of publications of the model categories published between 1998 and 2019. (C) Within the autologous transplantation model, tissue fragments may be implanted in different areas. Dark blue fraction: peritoneum, blue fraction: abdominal wall, cyan fraction: mesenteric arteries/vessels (A/V), light blue fraction: ovary, white fraction: colorectal region, lilac fraction: other regions, and gray fraction: not specified. (D) Scientific articles published per year using the autologous transplantation model between 1998 and 2019. (E) Variants of the syngeneic transplantation model. Dark turquoise fraction: publications using intraperitoneal injection of suspended tissue and turquoise fraction: publications using suture. Light turquoise fraction: publications using subcutaneous injection of suspended tissue. Gray fraction: articles where it was not specified. (F) Publications per year using the syngeneic transplantation model between 1998 and 2019. (G) Variants of the xenograft transplantation model. Dark green fraction: intraperitoneal injection of suspended tissue. Green fraction: suture of endometriotic tissue. Light green fraction: subcutaneous injection of suspended tissue. (H) Publications per year using the xenograft transplantation model between 1998 and 2019. (I) Allogeneic transplantation model variants. Brown fraction: peritoneum. Mustard fraction: mesenteric arteries/vessels (A/V). Light brown fraction: abdominal wall. Dark yellow: not specified. Gray: subcutaneous injection. (J) Publications per year using the allogeneic transplantation model between 1998 and 2019. N/S, not specified.

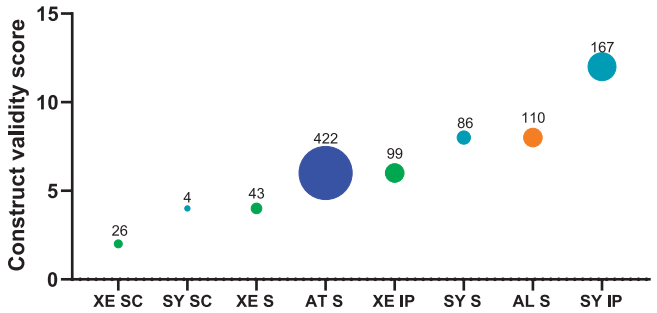

Figure 3. Construct validity for translational research of preclinical models of endometriosis. Based on the score results from our 3-point scale algorithm specifically developed to determine the construct validity for translational research purposes. The size of the dots represent the number of publications within the model, which are indicated on top of each dot as well. Colour of the dots indicates the model category; blue: autologous transplantation, green: xenograft transplantation, turquoise: syngeneic transplantation, and brown: allogeneic transplantation. The endometriosis model with the highest construct validity was syngeneic transplantation of endometriotic tissue by intraperitoneal injection of suspended tissue. AL-S, allogeneic transplantation models, suture; AT-S, autologous transplantation model, suture; SY-IP, syngeneic transplantation model performed by intraperitoneal injection; SY-S, syngeneic transplantation model performed by suture; SY-SC, syngeneic transplantation model performed by subcutaneous injection; XE-IP, xenograft transplantation model performed by intraperitoneal injection; XE-S, xenograft transplantation model performed by suture; XE-SC, xenograft transplantation model performed by subcutaneous injection.

perception cannot be reported by rodents, surrogate measures based on altered behaviours and/or altered response to stimuli are used.[125,126] The model subcategory having the biggest proportion of publications addressing pain is autologous transplantation with 12%, followed by syngeneic 10%, allogeneic 6%, and xenograft transplantation with 0.5%. Within the autologous transplantation model, for example, a model of viscero-visceral hyperalgesia was developed. In this model, female rats with previously induced ENDO were also subjected to ureteral calculosis. Spontaneous pain behaviour was observed for several days in rats having ENDO and stone implantation, in contrast to ENDO alone, or sham ENDO controls.[43,60,82] This model provides better insights into preclinical models regarding the occurrence of viscero-visceral hyperalgesia that also occurs in ENDO patients. However, it is unclear whether or not the reported observations may be due to the surgical induction of ureteral calculosis. From all publications studying nociception and pain, 87% used evoked-pain measures. By contrast, only 17% of publications measured nonevoked pain readouts. Within subcategories, the syngeneic transplantation model variant had the highest number of publications studying nonevoked pain: 11 publications accounting for 42% of the publications addressing pain in this subcategory.[13,36,47,73,74,106] Sub/infertility, another common symptom reported by women with ENDO, was also surprisingly understudied, accounting for up to only 1%–6% of publications from all model subcategories.

3.4. Preclinical models of interstitial cystitis/bladder pain syndrome

In the last 22 years, the majority of preclinical models of IC/BPS were bladder-centric models, comprising 894 publications and representing 85% of the total. This was followed by models with complex mechanisms, which with 86 publications comprise 8% of total. Finally, the category of stress-induced models with 69 publications accounted for 7% of total (**Fig. 5A**). Because IC/BPS is a disorder occurring in both men and women, these results include publications with both male and female rodents.

For each IC/BPS model category, the number of articles published per year was analysed (**Fig. 5B**). The largest model category, bladder centric, was also the first methodology to be established, and publications using this model increased almost continuously every year until 2016, reaching a peak of 71 publications, followed by a plateau and subsequent decrease in 2019 with only 33 publications (**Fig. 5B**). In the bladder-centric category, 3 methods have been established: instillation of irritants (92% of publications), altered expression of urothelial targets (5% of publications), and radiation-induced cystitis (3% of publications, **Figs. 5C and D**). Instillation of irritants consists of infusing the bladder with an agent that damages the urothelium, generally achieved by transurethral catheterization under anaesthesia. Irritants commonly used are as follows: acetic acid,[66] protamine sulphate,[38] and lipopolysaccharide.[83] This category also includes the cyclophosphamide model which, in contrast to the other irritants, is administered systemically. This model variant accounts for up to 48% of all publications within the category (426 publications, **Figs. 5C and D**). Within the subcategory "altered expression of urothelial targets," different techniques have been used aiming to understand the role of gene products in disease development, including gene therapy, transgenic mice, siRNA, and exogenous administration of specific peptides or protein inhibitors.[22,29,64,68,98] The subcategory "radiation" consists of specifically irradiating the bladder one or more times, producing local tissue damage, and triggering haemorrhagic cystitis.[136]

Within the "complex mechanisms" category, 5 different subcategories to induce bladder complications have been identified (**Figs. 5E and F**): autoimmune model, colonic instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS), injection of pseudorabies virus in the tail, pelvic pain comorbidities, and others. The autoimmune model accounted for 43% of publications within its category, with that being the biggest subcategory, followed by colonic instillation of TNBS. Colonic instillation of TNBS induces local inflammation and consequently bladder complications which are generally measured between 3 and 30 days after instillation.[63,85,130] This subcategory accounted for 20% of publications in the category. Another model variant consists of injecting pseudorabies virus in the tail of the animals, inducing neurogenic cystitis characterized by progressive pelvic pain. The outcomes from model induction are generally measured 2 to 5 days after injection.[21,113,142] This subcategory accounted for 14% of publications in the category. The next subcategory, pelvic pain comorbidities, includes experimental procedures in which other pelvic areas were impaired, development of bladder complications, eg, prostatic inflammation,[97,116] constipation,[58] or uterine pain.[76] Bladder complications are measured between 7 days and 4 weeks after model induction. This subcategory accounted for 8% of publications. Finally, all other complex mechanisms that could not be related to any of the former subcategories were grouped together, eg, intake of tranilast, an antiallergy agent that has been reported to lead occasionally to IC/BPS in patients[99]; and systemic administration of peptides/proteins that modulate the sensory nervous system and/or brain function.[18,89,118] Together, this subcategory accounted for 15% of publications. In addition, publications using these techniques have shown a cyclic pattern with 3 different peaks of 6, 7, and 9 publications in the years 2007, 2012, and 2016, respectively, followed by a plateau and decrease in the respective following years (**Fig. 5F**).

Finally, stress-associated IC/BPS models are the newest and least used models, being first described in 1999 and becoming

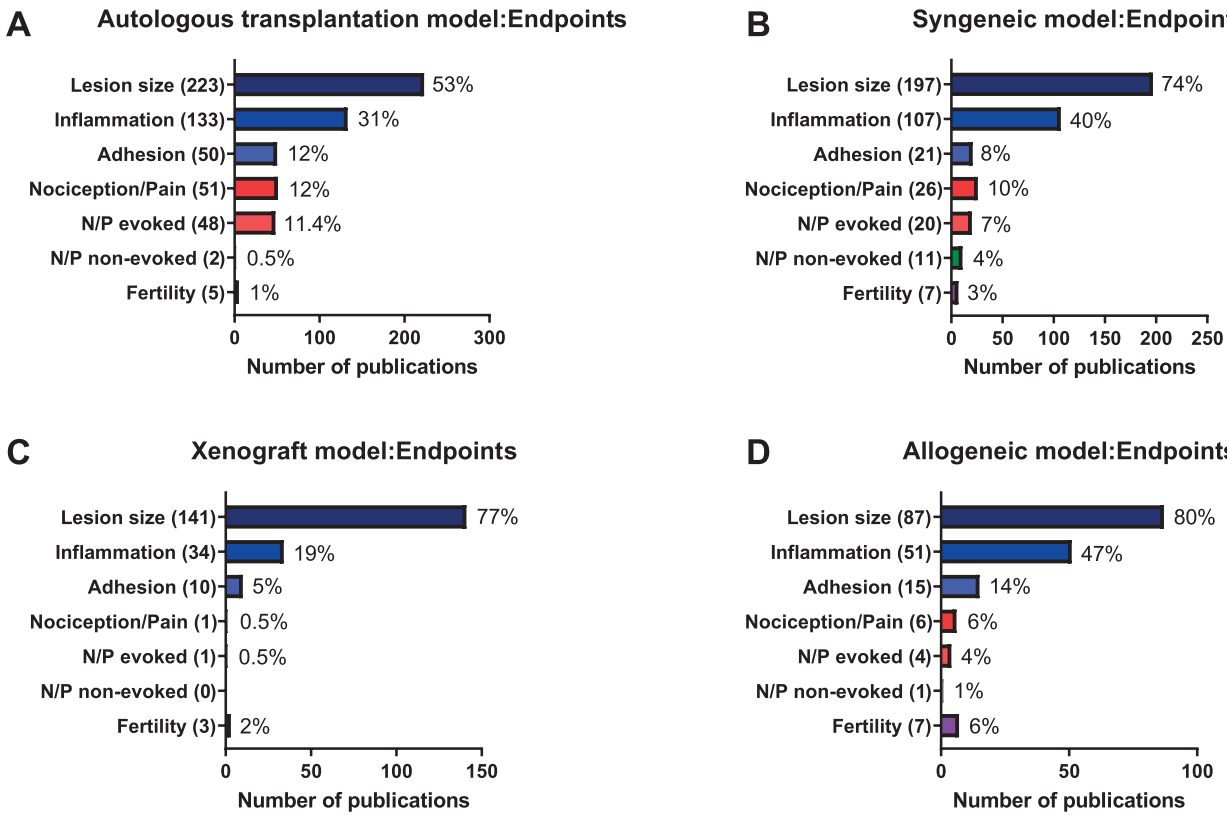

Figure 4. Face validity of preclinical models of endometriosis. Analysis of the outcomes measured in the publications within each model category. (A) Autologous transplantation model. (B) Syngeneic transplantation model. (C) Xenograft transplantation model. (D) Allogeneic transplantation model. Dark blue bar: percentage of publications that measured macroscopic changes in either lesion size, volume, weight, or number. Blue bar: percentage of publications that measured of inflammatory markers. Light blue bar: percentage of publications that quantified adhesions. Purple bar: percentage of articles that measured fertility. Red bar: percentage of publications that assessed nociception/pain aspects. Coral bar: percentage of publications that assessed evoked-pain readouts. Green bar: percentage of publications that assessed nonevoked pain readouts. N/P, nociception/pain.

increasingly used as from 2006. The peak was reached in 2013 with 10 publications (**Figs. 5G and H**). Within the stress-induced model category, 5 different methods have been identified: water avoidance stress, manipulation of the environment, footshock stress, neonatal maternal separation, and restrainment stress. The most published model variant is water avoidance stress, which consists of subjecting the animals to either acute (1 day) or chronic (10 days) stress by placing the animals for 1 hour on a platform inside a container full of water. Generally, bladder complications are measured one day after the last day of the test.[91,107] This subcategory accounted for 39% of publications, followed by manipulation of the environment. In the latter, the model variant may be induced by chronic variable stress,[51] early life stress by odour-shock conditioning,[78] cold stress,[59] or social stress.[96] Outcomes were measured in adulthood and vary depending on the stress protocol. This subcategory accounted for 32% of publications within stress-induced model subcategories. Next, the subcategory footshock stress accounted for 13% of publications and consisted of performing an electric foot shock of 1 mA for 1 second, the animals afterwards being subjected to functional experiments.[112] The subcategory restrainment stress accounted for 10% of publications within stress-induced models and consisted of immobilizing the animals in a restrainer for 3 to 4 hours[19,35] before measuring outcomes in bladder morphology and/or function. Finally, the subcategory neonatal maternal separation consisted of separating animal litters from their mother on postnatal days 1-5 to 21-25 daily for 3 hours. Subsequently, bladder complications were measured when animals reached 1 to 5 months of age.[90,108] The number of publications reporting this model accounted for 7% of publications.

3.5. Face validity in preclinical models of interstitial cystitis/bladder pain syndrome

In IC/BPS patients, several clinical features can be observed: bladder pain, micturition frequency, nocturia, perineal pain, glomerulations (although they are not IC/BPS specific), Hunner lesions, and urothelial denuded areas. These aspects have been mimicked partly in preclinical models of IC/BPS. In this study, we have categorized them as follows: "bladder morphology," "bladder function," "behaviour," and "nociception/pain"; the latter being further classified as "evoked" and/or "nonevoked," and finally "others" (**Fig. 6**).

The endpoint "bladder morphology" indicates that the microscopic bladder structure has been analysed. This allows evaluation of the degree to which the clinical hallmarks "glomerulations" and "Hunner lesions" are present in the animal model by detecting inflammatory markers in bladder tissue and areas of urothelial denudation. Likewise, with the endpoint "nociception/pain," it is possible to evaluate to what degree bladder and perineal pain are seen in the animal models. Finally, with the endpoint "bladder function," both nocturia and micturition frequency can be evaluated.

The most addressed endpoint was bladder function, being analysed in 55% of publications using bladder-centric models

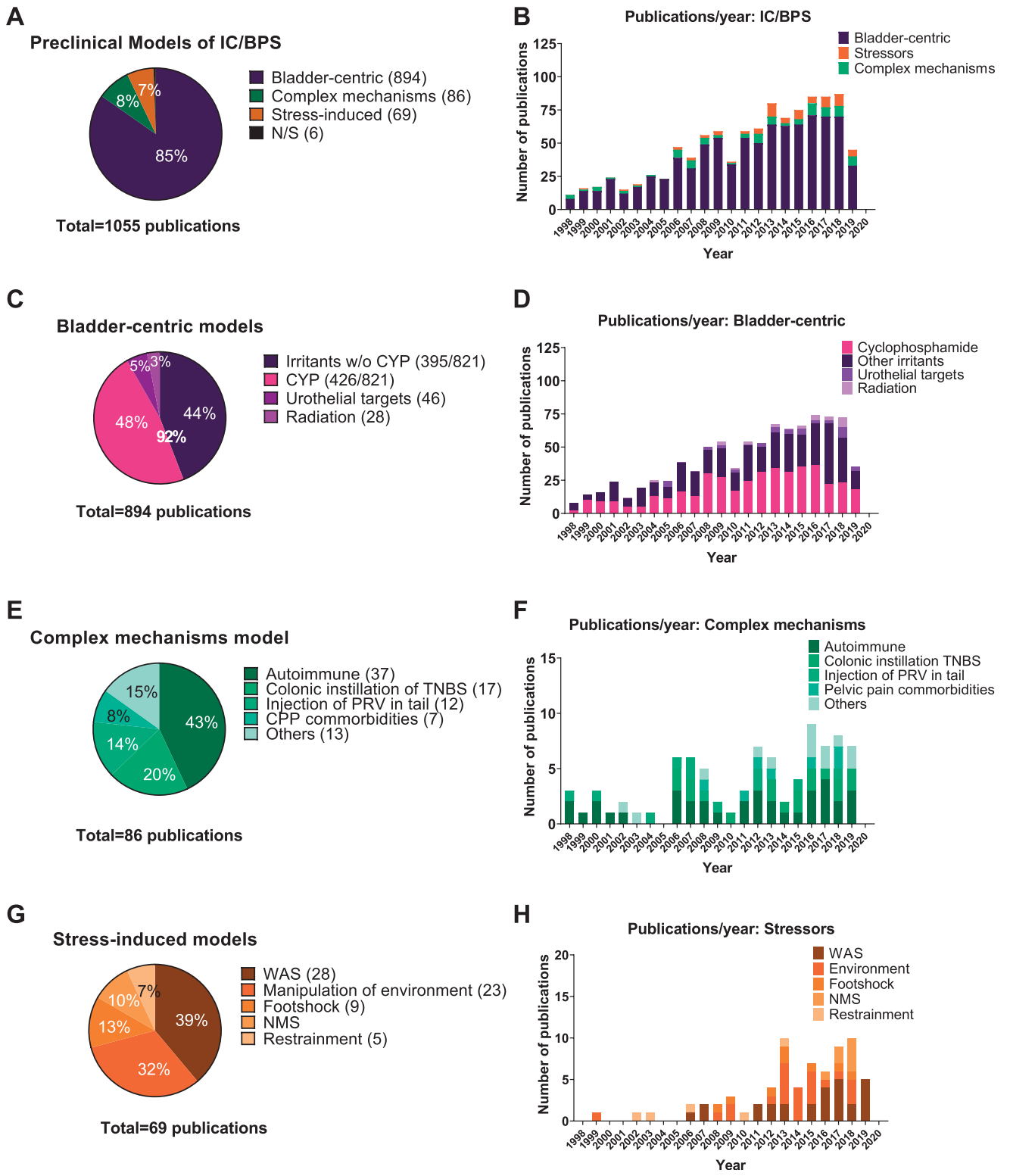

Figure 5. Preclinical models of IC/BPS. (A) Preclinical models of IC/BPS. Three main categories were found. Dark purple fraction: bladder-centric models. Green fraction: complex mechanisms models. Orange fraction: stress-induced models of IC/BPS. (B) Number of publications of the model categories published per year during 1998 to 2019. (C) Variants of the bladder-centric model. Dark purple and fuchsia fractions: instillation of irritants. In fuchsia, the use of cyclophosphamide (CYP) is highlighted. Purple fraction: models with altered expression and/or function of urothelial targets. Light purple fraction: radiation-induced cystitis. (D) Publications per year using bladder-centric model variants between 1998 and 2019. (E) Variants of the complex mechanisms model. Dark emerald fraction: autoimmune models. Emerald fraction: colonic instillation of TNBS. Light emerald fraction: injection of PRV in tail. Mint fraction: chronic pelvic pain (CPP) comorbidities. Light mint fraction: others. (F) Publications per year using complex mechanisms models between 1998 and 2019. (G) Variants of the stress-induced model. Dark orange fraction: WAS. Orange fraction: manipulation of the environment. Light orange fraction: footshock stress. Sand fraction: Neonatal maternal separation (NMS). Light sand fraction: restrainment stress. (H) Publications per year using stress-induced models between 1998 and 2019. IC/BPS, interstitial cystitis/bladder pain syndrome, PRV, pseudorabies virus; TNBS, 2,4,6-trinitrobenzenesulfonic acid; WAS, water avoidance stress.

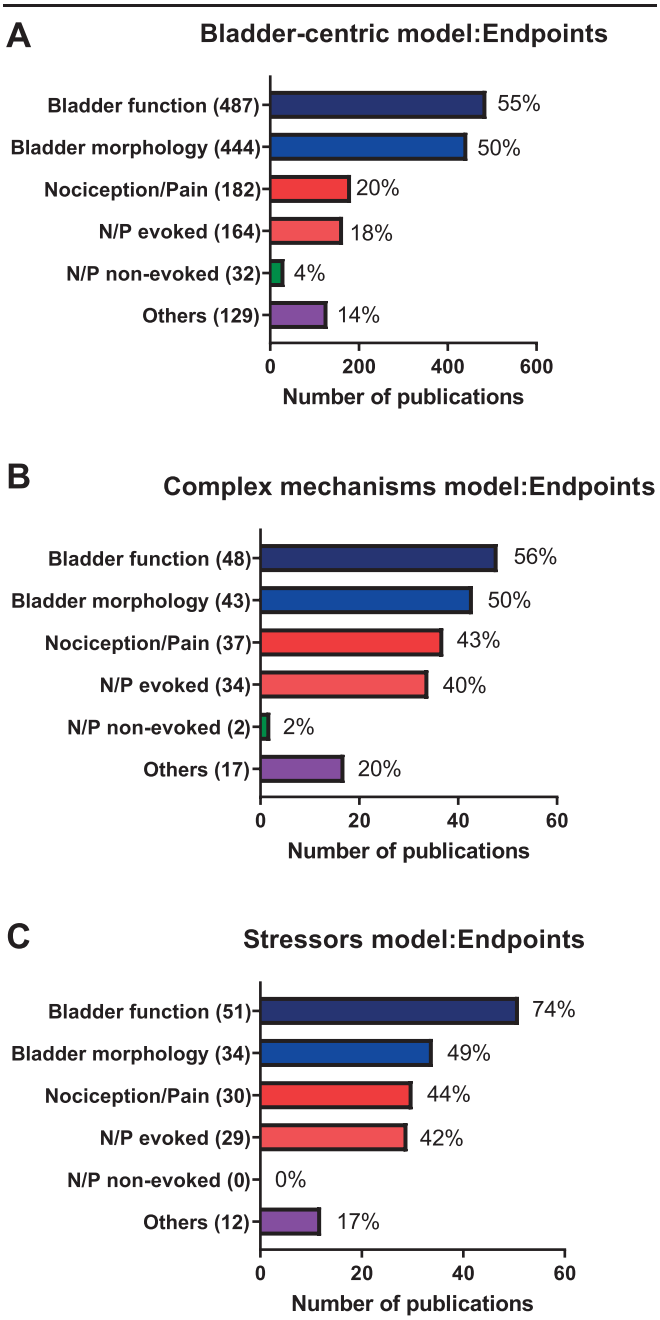

Figure 6. Face validity of preclinical models of IC/BPS. Analysis of the outcomes measured in the publications within each IC/BPS model category. (A) Bladder-centric models. (B) Complex mechanisms models. (C) Stress-induced models. Blue bar: percentage of publications that assessed bladder function by cystometry, voiding spot, or metabolic cages. Light blue bar: percentage of publications that assessed bladder morphology by histopathology, bladder weight, or immunohistochemistry. Red bar: percentage of publications that assessed nociception/pain endpoints. Coral bar: percentage of publications that assessed evoked-pain readouts, such as abdominal von Frey or VMR. Green bar: percentage of publications that assessed nonevoked pain readouts, such as observing pain-related behaviours among individuals during a definite time frame. Purple bar: percentage of publications that assessed other endpoints such as changes in expression of different markers in tissues other than the bladder. IC/BPS, interstitial cystitis/bladder pain syndrome; N/P, nociception/pain; VMR, vasomotor reflex.

(**Figs. 6A**), 56% of publications using complex mechanisms models (**Figs. 6B**), and 74% of publications using stress-induced models (**Fig. 6C**). The second most evaluated endpoint was bladder morphology, which was evaluated in 50% of publications using bladder-centric models, 50% of publications using complex mechanisms models, and 49% of publications using stress-induced models. Endpoints belonging to the category "others"—including gene expression/function in tissues other than the bladder or nonstructural gene expression analyses—were evaluated among categories almost in the same proportion, varying between 14% and 20% of total publications within each subcategory.

Nociception and pain endpoints were evaluated in a subset of publications investigating preclinical models of IC/BPS. The model subcategory most covering these outcomes is the stress-induced model subcategory with 44% of total publications, followed by complex mechanisms with 43% of publications, and finally bladder-centric models with 20% of publications. In other words, the largest model subcategory is also the one with the smallest proportion of studies including nociception and/or pain endpoints and vice-versa. However, of all publications studying nociception and pain within IC/BPS model categories, 91% used evoked-pain measures, and only 14% measured nonevoked pain readouts. Within subcategories, "bladder centric" was the model variant with the highest number and proportion of publications studying nonevoked pain: 32 publications accounting for 13% of the publications addressing pain and 4% of the total publications in this subcategory.

4. Discussion

Recently, the World Health Organisation recognised chronic pain as a disease in its own right. Within this framework, ENDO and IC/BPS are categorised as "chronic visceral pain from persistent inflammation in the pelvic region," as described in the International Classification of Diseases 11th.[6] The causes of both diseases remain unknown; there is no cure, and pain frequently persists and may worsen.

In this comprehensive literature review of all publications in the last 22 years using preclinical models of ENDO and IC/BPS in rodents, we have analysed which translational features have been studied more or less frequently to uncover aspects of high relevance to patients who have not, or been insufficiently, evaluated and need to be addressed to improve the development of new therapies for both conditions. In summary, our results indicate:

(1) The most frequently used preclinical model of ENDO is autologous transplantation, followed by syngeneic transplantation. Although the former seems to be declining in use, the latter has been increasingly used across the years. By contrast, the most frequently used preclinical models of IC/BPS were bladder centric. However, the number of publications in the last 3 years for bladder-centric models has been on the decline, whereas other model categories, such as complex mechanisms and stress-induced, are gaining traction.

(2) By adopting a 3-point scale to evaluate the construct validity of preclinical models of ENDO in the published literature, we found that syngeneic transplantation of endometriotic tissue, specifically performed by intraperitoneal injection, resembles the physiopathology of ENDO in a more reliable manner than the other subcategories. Unfortunately, with the current lack of knowledge of disease aetiology, it was not possible to develop a construct validity scale for preclinical models of IC/BPS as it remains unclear with which pathomechanisms the models need to be compared.

(3) In both pelvic pain disorders, the face validity has mostly been restricted to demonstrating tissue damage and organ dysfunction, despite the fact that patients suffer from CPP and other disease-associated traits (in ENDO: fertility issues, in IC/

BPS: urinary urgency). In addition, when assessing pain, preclinical models mostly used evoked pain measures rather than measuring ongoing pain. However, the latter is a significant element of the clinical symptoms of both disorders.

4.1. Rationale for the construct validity scale for translational research

Although all preclinical models of ENDO have been helpful in improving our understanding of lesion formation and disease progression, they also have a number of limitations when considering their relevance for translation to patients. We developed a scoring system for preclinical models of ENDO specifically designed to highlight which models are more suitable for translational research purposes. The scoring system is based on 3 criteria: first and foremost, it attempts to evaluate how closely each model mimics the pathomechanism of ENDO. Then, it aims to evaluate in each model to what degree the immunoinflammatory components of the animal model can be studied. Finally, it aims to evaluate which models have the ability to study the function of specific gene products.

Surgical attachment of endometriotic tissue fragments is routinely performed in all transplantation model subcategories. This technique offers the advantage of choosing the areas for disease establishment and progression. Examples of such areas include the ovaries, mesenteric arteries/vessels, or colorectal region.[42,52,143] However, surgical implantation is a highly invasive procedure that encompasses administering general anaesthesia, opening and exposing tissue layers in the pelvic area, and suturing tissue implants. This procedure unavoidably induces postoperative pelvic pain, as well as a risk of infection. Nevertheless, when comparing allogeneic vs autologous transplantation, the advantage of autologous transplantation, which is usually performed in rats, rather than allogeneic transplantation, which is performed in mice, is that the size of the animal allows for surgery, that is, relatively easy to perform. In the allogeneic transplantation model, because mice are smaller, performing the surgery can be more challenging although the availability of large numbers of transgenic lines of mice opens up the possibility of studying the role of specific genes and cell types in the development of the disease.[34,93]

In contrast to surgical procedures, injection of suspended tissue provides a considerably less invasive way of delivering endometriotic tissue without causing external or internal tissue injury. Specifically, intraperitoneal injection resembles more accurately what would occur during retrograde menstruation in humans. Retrograde menstruation is the physiological process involving the backward flux of menstrual debris containing viable endometrial cells through the fallopian tubes into the pelvic cavity. This process occurs naturally in over 90% of women of reproductive age with patent fallopian tubes and provides the material to potentially develop ENDO.[145] However, because only a fraction of patients with retrograde menstruation will develop ENDO, additional factors must be involved in the establishment and development of lesions that underpin disease progression. These factors likely include endocrine and metabolic (pelvic) environment, epithelial–mesenchymal transition, and altered immunity and inflammatory responses in those genetically susceptible.[145]

Comparing xenograft and syngeneic transplantation models, the 2 models that have used intraperitoneal injection of suspended tissue, xenograft transplantation models are developed by transplanting human endometriotic tissue or cells directly from patients, bringing with them all the disease factors that are actually present within them. The disadvantage of this model is that for mice to receive tissue of human origin, immunosuppression is required. Because ENDO is considered to be an estrogen-dependent inflammatory disorder, the contribution of inflammatory components of the recipient mice can only partially be studied. Nude mice have deficits in their reproductive system, including reduced circulating hormone levels.[111] Furthermore, because of the lack of T lymphocytes and/or B lymphocytes, depending on the phenotype, they are more susceptible to pathogens, and special care must be taken when performing behavioural studies. Most strikingly, in a model of colorectal pain, nude mice have shown abnormal pain sensation which is normalized after supplementation with T cells, demonstrating the link between T lymphocytes and visceral pain sensitivity.[135]

4.2. Technical considerations on the syngeneic transplantation model performed by intraperitoneal injection model variant

Between research groups performing this model variant, differences in the methodology are apparent. For example, after extracting and splitting the uterus horn from donor mice, some groups report cutting the tissue fragments or mincing the uterine tissue to obtain tissue fragments,[2,77,81,120,124,139] whereas others first remove the serosa layer and myometrium and use the remaining tissue as a source of tissue fragments for injection,[7,33,46,53,55,104,114] and others have reported squeezing the uterine horn to obtain endometriotic tissue.[27] These differences in experimental protocols might seem slight but, in fact, provide recipient mice with different amounts and ratios of cell types. Another example of variation is to regulate the hormonal cycle of the animals and to induce menstruation in mice. These protocols include one or more of the following procedures: performing ovariectomy in mice to suppress the endogenous hormone activity, administrating donor mice with oestrogen (17β-oestradiol) for a period of time (usually 7 days), administering progesterone in donor mice to induce decidualisation before tissue harvest, as well as supplementing recipient mice with oestrogen before and/or after intraperitoneal injection of endometriotic tissue fragments. These artificial hormonal interventions in rodents might have positive effects in construct validity, but only when carefully calibrated to replicate menstruation as well as the hormone fluctuations that occur in ENDO patients. It is important to note that because rodents do not menstruate naturally, intraperitoneal injection of suspended tissue fragments from cycling mice may therefore introduce a different mix of cells to those introduced by retrograde menstruation in women.

In this regard, a new variant of the syngeneic transplantation was established in 2014 in which menstrual tissue was generated in and collected from donor mice and injected intraperitoneally in recipient mice.[46] Generation of menstrual tissue for use with the syngeneic mouse model of ENDO involves the use of donor mice which undergo a protocol of artificial decidualisation and progesterone withdrawal to induce menstruation.[46] Specifically, donor mice are ovariectomised to deplete endogenous steroid hormones and then treated with a hormone regime that mimics the normal menstrual cycle of women.[46] Decidualisation occurs by intrauterine oil administration which simulates blastocyst attachment followed by withdrawal of progesterone, which results in endometrial breakdown.[46] The “menstrual” tissue is injected into recipient mice, and progression of ENDO can be monitored. It is important to note that although this model resembles the physiological processes believed to be involved in

lesion formation as a consequence of retrograde menstruation, it also entails the added complexity of inducing artificial menstruation in donor mice, which needs to be taken into account. Both the frequency and the extent of decidualisation in donor mice can vary between experimental runs, with anecdotal evidence suggesting that stress levels of mice may have an impact on decidualisation. In addition, the decidualised endometrium is minced and injected into recipient mice, with no control over which tissue fragments will attach to form lesions, meaning that the cellular composition of the tissue fragments which will end up forming lesions can vary between animals, attachment sites, and experimental runs. Although this can be a drawback regarding controlled reproducibility, it does better resemble the lesion heterogeneity observed in those with ENDO. Interestingly, the established lesions in these mice demonstrated several hallmarks of the human disease, including lesion components, changes in oestrogen receptor expression, as well as macrophage infiltration and inflammation. Along the same lines, a new model of retrograde menstruation in rats was recently developed,[105] with promising insights into behavioural assessments of ongoing pain.

We recommend careful revision and discussion of these protocols, aiming to reach a consensus within the scientific community to improve understanding of the range of physiological parameters involved in these models. We hope to obtain greater insights into this regard in our IMI-PainCare project through controlled comparison of experimental protocols and performing multicentre staff training towards validated methods and comparable standard operating procedures.

4.3. Construct validity scale for preclinical models of interstitial cystitis/bladder pain syndrome

Interstitial cystitis/bladder pain syndrome is a difficult condition to diagnose, and there is limited understanding of the disease mechanism. Moreover, global consensus on definitions and treatment possibilities has not yet been achieved, and standard terminology is constantly evolving.[138] Based on this, it was not possible to establish a construct validity scale for IC/BPS. Given that the patient population is very heterogeneous in terms of detectable pathological changes, as well as local extension of the disease and comorbidities, we recommend that research focuses its efforts on deep patient phenotyping procedures. Patient phenotyping and subgrouping are likely to be essential to unlock major advances in the state of the art. Patients report a variety of different symptoms for both pain and urinary symptoms: In some patients, symptoms extend beyond the bladder, and in some, the disease is accompanied by different comorbidities. The multiplicity of symptoms and signs will require the use of different and appropriate animal models to progress translational studies of IC/BPS. In addition to the pain or the micturition phenotype, the molecular signature obtained from the analyses of urine, blood, or saliva samples of the patients will help to choose more appropriate animal models to address the putative diverse pathophysiological mechanisms of IC/BPS, pointing us towards to effective diagnostic tools and treatments.

Most of the bladder-centric models have limitations in reflecting the chronicity and comorbidities of IC/BPS. For example, models in the subcategory "instillation of irritants" generally induce an acute, short-term effect, and therefore, these experiments are often performed terminally. So far, there is no cure for IC/BPS,[30] which means that patients have to learn to live with the symptoms throughout their life, often with a tendency to accumulate comorbidities with time.[110] It will be worth refining the preclinical models to obtain more insights into these aspects, eg, by modulating the strength and duration of a trigger to induce a potentially milder but chronic disease state. A surprisingly high proportion of publications within the instillation of irritants subcategory used the cyclophosphamide model (48% of publications). Cyclophosphamide, which was originally administered in cancer patients as a chemotherapeutic agent, is metabolised into acrolein/ifosfamide, which accumulates in the bladder and in turn induces irritation of the urothelium, inflammation, and pain,[31,71] whereas it may also irritate many other tissues. In contrast with the rest of the irritants in the model category, this model variant can be administered systemically (generally injected intraperitoneally), which might explain why it has been preferred over the classic transurethral catheterization under anaesthesia commonly used for the rest of the irritants in the subcategory. In 2014, a chronic cyclophosphamide model was characterized,[45] and these mice, after repeated systemic injections of cyclophosphamide, have shown signs of mild inflammation, urothelium hyperplasia, and local changes in metabotropic glutamate receptor expression. However, these traits do not entirely represent what occurs in the bladder of IC/BPS patients, where fibrosis rather than hyperplasia may be observed in the urothelium of some patients,[12,65] and it is not clear to what extent these local changes in expression of glutamate receptors play a role in IC/BPS patients. Other traits that may occur in the cyclophosphamide model, such as increase in urinary frequency and/or urgency, can also be attributed to haemorrhagic cystitis, rather than IC/BPS. Therefore, the chronic cyclophosphamide administration model has limitations for translational research on IC/BPS.

A new bladder-centric model has been proposed as an alternative to normal irritants. This model is based on the breakdown of the urothelium's apical cell layer because of disruption of the glycosaminoglycan layer by enzymatic digestion.[100] The authors have presented 2 protocols of enzymatic deglycosilation: acute and chronic. They have found that after 2 hours of enzymatic digestion of the extracellular matrix of the urothelium, the mice replicate various pathological phenotypes found in patients as follows: disruption of the mucosal barrier with loss of the urothelial cell plaques, macrophage infiltration, increased urinary frequency and urgency, suprapubic hyperalgesia, and increased nociceptive activity.[100] A potential limitation of this model is that the effects induced by enzymatic digestion are reversed 7 days after model induction because of tissue repair which unfortunately does not occur in IC/BPS patients.

Acknowledgment of the limitations of the bladder-centric models in comparison with the complex clinical situation has probably driven the emergence of the complex mechanism models as well as the stress-induced models, as they apply stimuli beyond the bladder and thus shift the focus from the bladder to a more systemic origin of the disease, making it impossible to reliably score construct validity. Autoimmune models acknowledge the fact that in a significant number of patients IC/BPS occurs in association with autoimmune diseases such as Sjögren syndrome, rheumatoid arthritis, lupus erythematosus, and others.[11,79,131] In this model variant, an autoimmune response is triggered in the animals by injecting immunogen agents and/or homogenate of bladder tissue combined with complete Freund's adjuvant. Bladder function and morphology are assessed between 10 days and 4 months after induction.[10,62,144] The bladders from these animals show changes in both bladder tissue composition as well as bladder function, compared with control animals.[79]

Inside the complex mechanism category, chronicity of the models varies greatly, lasting up to 16 weeks,[122] with most of the models measuring their endpoints 1 to 12 weeks after model induction.[3,62,67,79,80] In the same way, within the stress-induced model category, chronicity varies between 8 and 12 days[1,40,91,137] and up to 8 weeks of age.[108] These long-term effects on the animals after model induction could be advantageous in construct validity for translational research; nevertheless, evaluation has to distinguish between acute and chronic effects, and those outcomes should then be carefully compared with patient outcomes. Nevertheless, these model categories might reflect the clinical situation only partially, and ultimately, it is unclear whether the pathophysiological alterations induced artificially in the animal models mimic the origin of the human disease. Moreover, the clinical situation does not easily allow separation of origin and consequence. Although there is evidence that traumatic experiences may be part of the disease history/origin in some patients,[24,28,92] it is known that stress and psychological perturbations also occur as a consequence of a disease which has a significant psychosocial impact on daily life, may reduce quality of life, impair sleep, and lead to depression.[70,132]

4.4. Face validity of preclinical models of endometriosis and interstitial cystitis/bladder pain syndrome

In over 2 decades of research using preclinical models of ENDO, most scientific articles (between 53% and 80%, depending on the model subcategory) measured the lesion size in their animal models as a primary endpoint. This endpoint has been used both as a proof-of-principle as well as to evaluate the effectiveness of a potential treatment. By contrast, only 0.5% to 12% of these scientific publications measured pain outcomes, and only 1% to 6% have measured fertility. This is a striking observation, considering that the most common symptoms of ENDO in patients are pain and infertility.[15] Moreover, the lesion size does not correlate with pain levels and fertility.[127,134] In IC/BPS, the most measured aspects in preclinical models were bladder function and bladder morphology, whereas measures of nociception and/or pain were only included in 20% to 44% of publications. Most strikingly, nonevoked pain outcomes comprised 0% to 4% of publications within the categories. Therefore, we highly recommend that the scientific community pays more attention to these aspects when pursuing development of new medicines, especially regarding pain-related outcomes.

Pain symptomatology in patients suffering from these chronic pain conditions is diverse: In ENDO, up to 80% of patients suffer from cyclic dysmenorrhea (pain during menstruation), up to 50% suffer from CPP, 40% to 50% suffer from deep dyspareunia (pain during intercourse), and a small percentage of patients (1%-2%) suffer from dysuria (pain during urination), and/or dyschezia (rectal pain during bowel motion or in some patients because of rectal bleeding during menstruation).[15,115] In IC/BPS, the most severe pain symptoms are bladder pain and overall pain, pelvic pain, abdominal pain, bloating pain, dysmenorrhea, back pain, pain during bladder filling, and dyspareunia.[26] With sustained pain over time, central sensitization is also a phenomenon occurring in patients with ENDO[48] and IC/BPS.[69,102] Both somatic and visceral pain should be studied in detail in preclinical models of ENDO and IC/BPS to refine them for improved face validity. Importantly, neurogenic inflammation should also be considered as a possible mechanism in the development of both conditions. It has been proposed that retrograde menstruation is the first step in the development of ENDO-associated pain75. In this theory, ENDO-associated pain (EAP) would occur through local release of proinflammatory mediators by the same sensory neurons that respond to the presence of pronociceptive mediators. These, in turn, sustain the presence of immune cells, tissue degeneration, and release of more pronociceptive mediators, creating a positive feedback loop of sustained pain sensation, called neurogenic inflammation.[75] In IC/BPS, neurogenic inflammation may also manifest itself through inflammatory components in the bladder triggering pain sensation and sensitization of the sensory neurons innervating the bladder, contributing to chronic pain.

Pain research has evolved over the years, shifting from measuring physiological pain thresholds and determining hyperalgesia and allodynia to the study of chronic pain by measuring ongoing pain with nonevoked pain measures. Detection of pain thresholds and the existence of hyperalgesia and/or allodynia are helpful in the detection of acute and chronic pain states and also to help determine central sensitization. Nevertheless, these readouts may also exert stress and anxiety in animal models, and the lack of observation of these phenomena does not exclude that they do not exist but may be due to technical characteristics of the experimental design (eg, no difference in hyperalgesia levels between sham and verum after surgical induction of the model, in which the surgical intervention for both groups masks any difference in pain levels between groups), or even to the time when the test is performed. Nowadays, there is growing acknowledgement of the need for longitudinal studies to quantify and characterize the ongoing pain that occurs in chronic pain conditions.[126] In this regard, several alternatives to measure nonevoked pain have been proposed, including voluntary wheel running,[39,86,109] recording of ultrasonic vocalisations in rodents,[101,140] and recording spontaneous rodent behaviour in home-cage monitoring systems. In the latter, a few providers allow simultaneous recording of animals in social groups,[8,129] opening the possibility of analysing the social effects of pain in rodents, and investigating how these might translate to the social effects of pain in patients.

We argue that further development of these ongoing observations of pain-related behaviours are highly valuable for monitoring outcomes in preclinical models of chronic pain, especially for ENDO and IC/BPS, and would substantially increase their face validity for translational research. Therefore, these methodologies should be taken into account in the experimental design of preclinical models aiming to discover future therapies to treat and hopefully cure ENDO and IC/BPS, as well as improve the patient's quality of life.

As part of our collaborative work between consortium members, the section below includes the perspective of 3 partners representing patient organizations: Judy Birch (The Pelvic Pain Support Network), Lone Hummelshoj (endometriosis.org), and Jane Meijlink (the International Painful Bladder Foundation).

4.5. Patients' perspective on

4.5.1. Pelvic pain and endometriosis

This analysis demonstrates that pain has rarely featured in published articles which aim to establish an effective mouse model for developing treatments for the pelvic pain conditions: ENDO and IC/BPS. It seems from the analysis that there are more articles reporting on pain in IC/BPS than in ENDO, which is puzzling. It is hugely disappointing and surprising/shocking that the importance of pain has received relatively little attention in the

translational research field in these pelvic pain conditions, despite several decades of awareness raising by patients/patient representatives highlighting pain as a priority among clinicians, academics, researchers, policy makers, and the public. This is especially so in the case of ENDO which, despite having attracted increased publicity in recent years, is woefully underfunded in terms of research investment. Regretfully, there has been no consensus to date around a suitable model to develop urgently needed new treatments, addressing not only the pain symptoms associated with ENDO, but also other symptoms including infertility and fatigue. We hope this analysis together with developing a way of accurately assessing pain in rodents may be addressed by the IMI-PainCare TRiPP project ultimately leading to the development of more effective treatments for ENDO and IC/BPS.

4.5.2. Interstitial cystitis/bladder pain syndrome

Interstitial cystitis/bladder pain syndrome covers a wide spectrum of severity, varying from very mild to devastatingly severe and can be an immensely stressful disease. Its hallmark symptoms of pain or discomfort associated with an urgent and frequent need to void day and night can cause great stress, anxiety, and depression, with some patients afraid to leave their home for fear of not finding a toilet in time. The impact on work and (sexual) relationships causes further stress. Many patients say that getting IC/BPS not only changed their life but also their whole character. The fact that it may take years and many doctors to obtain the right diagnosis creates more anxiety and loss of self-confidence and may have a long-lasting detrimental psychosocial effect on the patient. Even in today's world, women's urogenital complaints are still often dismissed in Freudian style as hysteria, and this can lead to long delays in getting treatment.

Because of the fact that in recent decades many patients with unidentifiable bladder pain and urinary symptoms have been piled onto the miscellaneous, heterogeneous heap now known as IC/BPS, treatment continues to be trial and error, whereas many patients never find a successful treatment to relieve their symptoms. Finding an effective treatment for the individual patient will continue to be difficult without meaningful subtyping and phenotyping for both research and treatment purposes.

Many IC/BPS patients suffer from one or multiple comorbid diseases, thereby increasing suffering and making treatment even more complex. More research is needed into the relationship between these comorbidities and IC/BPS and to ascertain whether the bladder disorder forms part of a systemic autoimmune disease in some of these patients.

Where research is concerned, in recent years, too much attention has been focused on the evoked pain aspect alone to the exclusion of urgency and frequency symptoms—which in practical terms may cause the most bother in many patients—as well as nonevoked pain readouts. This has led many drug trials based on evoked pain to fail or to new drugs helping only very few patients. Research can therefore be improved by (1) taking all key symptoms into account including nonevoked pain readouts, sensory urgency,[94] and urinary frequency and b) phenotyping the patients. For clarification, the term "evoked pain" means the use of sensory stimuli to detect and quantify pain thresholds. By contrast, the term "nonevoked pain" means to record and quantify pain-related behaviour without applying sensory stimuli.

Preclinical research with rodents has tended to look at stress-induced pain behaviour, whereas little research has been performed into the stress and anxiety caused to the patient by the disease itself, by inadequate treatment, by a lack of understanding and indeed empathy regarding the consequences of the disease and by failure to take a serious look at what is needed to limit the impact of these consequences so as to improve the patient's (psychosocial) quality of life.

4.6. Future perspectives

Chronic pelvic pain/urogenital pain conditions as a whole are underresearched in comparison with other chronic pain conditions. The IMI PainCare with the subproject TRiPP comprises a large public–private partnership between the European Union and the European Federation of Pharmaceutical Industries and Associations. The TRiPP subproject aims to improve disease understanding of 2 specific conditions associated with CPP in women: ENDO because of its prevalence and IC/BPS, a less common condition but associated with a significant impact on quality of life.

The subproject TRiPP will be adopting new approaches to stratify patients by identifying underlying mechanistic pathways, leveraging cross-disciplinary knowledge of pain mechanisms with state-of-the-art biomarker discovery. We will implement a back-translational approach to these conditions, characterising the women suffering with these 2 forms of CPP in our clinical work package and then refining preclinical models of these conditions in light of these findings in our preclinical work packages.

For this purpose, the team is conducting one noninterventional trial (TRiPP NIT1) on deep phenotyping of women with EAP and IC/BPS and will use the findings to evaluate whether women with EAP and IC/BPS can be stratified and to back translate into more relevant preclinical models and improve the translational path to clinical studies.

Thus, our review focuses on these 2 conditions as relevant to this project. Although it would be interesting to expand these analyses of preclinical models of other CPP conditions and of BPS in males, this is beyond the scope of this project/review.

Conflict of interest statement

All coauthors are members of the IMI-PainCare consortium. A. Hoffmann, A. Laux-Biehlmann, B. De Leo, J. Nagel, P. Nunez-Badinez, and T.M. Zollner report personal fees from Bayer AG during the conduct of the study. A. Hoffmann, A. Laux-Biehlmann, B. De Leo, J. Nagel, and T.M. Zollner report personal fees from Bayer AG outside the submitted work. F. Barthas reports personal fees from Grünenthal GmbH during the conduct of the study and outside the submitted work. F. Cruz reports grants and personal fees from Allergan, personal fees from Astellas, personal fees from Bayer, and personal fees from Recordati, outside the submitted work. K. Vincent reports grants from Innovative Medicines Initiative during the conduct of the study; grants and personal fees from Bayer Healthcare, personal fees from Grunenthal GmBH, personal fees from Eli Lilly, and personal fees from AbbVie outside the submitted work. R-D. Treede reports grants from the IMI2 PainCare project of the European Union during the conduct of the study and personal fees from Bayer, Grünenthal, GSK, Sanofi outside the submitted work. J.D. Armstrong reports personal fees and others from Actual Analytics Ltd during the conduct of the study. The remaining authors have no conflicts of interest to declare.

Acknowledgments

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No [777500].

This joint undertaking receives support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations.
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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B319.

Article history:
Received 1 October 2020
Received in revised form 15 February 2021
Accepted 18 February 2021
Available online 3 March 2021

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