scaffold was added into the co-culture system, it showed simultaneous modulation of the inflammatory response in hMSCs and diseased HSFs through significant downregulation of ADAMTS-4, MMP-13 and CCL-2. Furthermore, the scaffold partly 'rescued' the impaired differentiation seen in hMSCs when co-cultured with diseased HSFs at 21 days, where the presence of the scaffold improved calcium (for osteogenesis) and proteoglycan (for chondrogenesis) deposition in the extracellular matrix (Fig 1).

Conclusions: The findings suggest that diseased cells arising from joint injury can create a highly inhibitory environment that increases inflammation in stem cells normally involved in the repair of musculoskeletal tissues, which impairs their regenerative ability. This inhibitory environment can be reversed by 'priming' the injured joint prior to treatment, such as through an intra-articular injection of hMSCs, which can help modulate the pro-inflammatory state of the resident joint tissues. A Sr-HT-Gahnite bioactive scaffold may complement the effects of hMSCs to further promote regeneration in the joint. This novel regenerative medicine approach of combining stem cells and a bioactive scaffold may be useful as a new therapy to treat chronic joint injury and reduce the risk of osteoarthritis progression.

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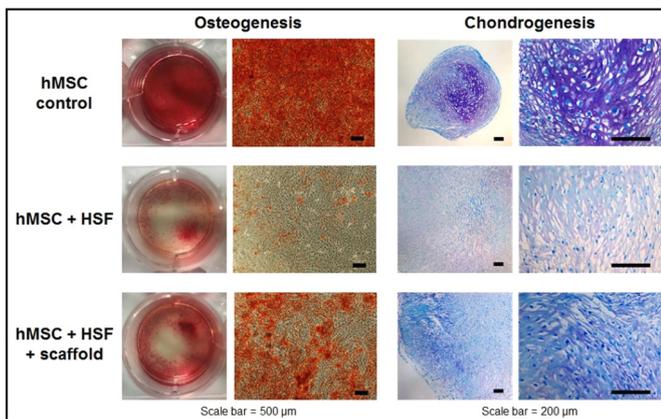
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THE MURINE EAR WOUND CARTILAGE SUPERHEALER TRAIT IS ASSOCIATED WITH GUT MICROBIOTA CHANGES AND IS TRANSFERRABLE TO NON-HEALER MICE BY GUT MICROBIOME TRANSPLANT

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Purpose: MRL/MpJ mice are substantially protected from developing post-traumatic osteoarthritis (OA), a trait with strong correlation to the ability to heal ear wounds. Previous studies have shown murine ear wound healing to be genetically determined. A powerful role of the gut microbiome in murine OA has been recently suggested, with dietary oligofructose supplementation resulting in protection from OA. In this study, we examined whether ear wound healing in MRL mice is associated with gut microbiota changes, and whether microbiome transplantation can transfer this superhealer trait to non-superhealer B6 mice.

Methods: In young mouse experiments, three-week-old C57BL6/J (n=26) male and female mice at weaning were given omeprazole 50mg/kg by oral gavage once daily for 3 days, then inoculated by oral gavage with diluted cecal contents from adult male or female MRL/MpJ mice resuspended in 1:1 PBS:glycerol once. Age-matched C57BL6/J (n=18) and MRL/MpJ (n=8) mice were gavaged with PBS:glycerol vehicle as controls. Three weeks later, all mice received a 2mm ear punch. In adult mouse experiments, 13-week-old C57BL6/J (n=7) male mice were given omeprazole for 3 days and inoculated with cecal contents from male MRL/MpJ mice once, as above. Age-matched C57BL6/J (n=4) male mice were gavaged with vehicle control. Three days later, mice received a 2mm ear punch. All mice were sacrificed 4 weeks following earpunch, final earhole size measured, and DNA extracted from cecal contents. The gut microbiome was characterized by 16s rRNA gene deep sequencing and analyzed using QIIME 1.9.1.

Group significance-values and FDR-corrected q-values were calculated in QIIME using Kruskal-Wallis and Benjamini-Hochberg. Clades characteristic of each group were calculated and plotted in LEfSe. Microbiome clades were correlated with earhole closure via Pearson correlation.

Results: As previously reported, MRL ear wounds closed well (MRL: 1.6 ± 0.1mm mean±SEM mm earhole closure vs. B6:0.23 ± 0.04, p<0.0001). Young transplanted B6 mice closed roughly one-half as well as MRL (MRL->B6: 0.9 ± 0.06 vs. MRL: 1.6 ± 0.1, p<0.0001), significantly more than B6 controls (p<0.0001), (Figure 1A). There were no significant differences in closure rates comparing male to female donors or recipients (Figure 1B). In adult mice, transplantation also resulted in improved wound healing, albeit of a lesser magnitude (MRL->B6: 0.60 ± 0.06 mean±SEM, mm earhole closure, vs. B6 vehicle: 0.25 ± 0.07, p=0.006) (Figure 1C). Microbiome analysis demonstrated several clades characteristic of each group (Figure 1D); specifically in MRL and transplanted mice, increases in Lachospiraceae (B6 mean OTU count 0.05 vs. transplant 4.4 vs. MRL 9.4, p=1E-5, q=0.003), Peptostreptococcaceae (B6 0.28 vs. transplant 0.46 vs. MRL 7.6, p=eE-4, q=0.02, and decreases in Coriobacteriaceae (B6: 10670 vs. transplant: 4982 vs. MRL: 795, p=0.0007, q=0.02) and Erysipelotrichaceae (B6: 0.33, transplant 3.2, MRL 2, p=0.002, q=0.05) were seen. The presence of several clades were highly correlated with earhole closure rates in individual mice, including Coriobacteriaceae (Pearson R=-0.53,p=3.5E-5), Figure 1E.

Conclusions: Superhealer MRL mice have substantial differences in gut microbiota composition compared to nonhealer B6 mice. The MRL cartilage healing trait, as measured by earhole closure, is partially transferrable to both weaning-age and adult B6 mice via a gut microbiome transplant and associated with shifts in specific gut microbiotic taxa. Future work should focus on elucidating the causal mechanism underlying these findings, and further examine the therapeutic potential of gut microbiota modification in treatment of OA.

Figure 1:
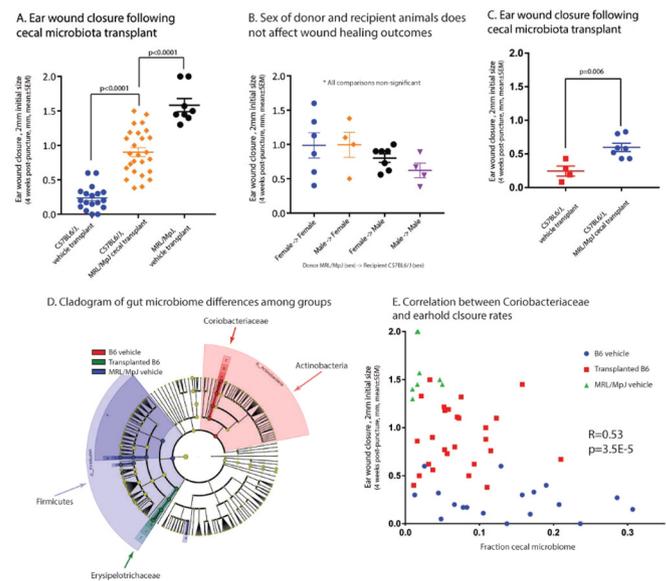
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IDENTIFYING PREDICTORS OF PLACEBO RESPONSE IN OSTEOARTHRITIS CLINICAL TRIALS OF THREE AGENTS WITH DIFFERENT ROUTES OF DELIVERY: A META-ANALYSIS USING INDIVIDUAL PATIENT DATA

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Purpose: Most drug treatments for osteoarthritis (OA) do not achieve a minimum clinically important difference above placebo and often associate with side-effects. On average, 75% of the analgesic effect from OA treatments in clinical trials, and potentially in clinical practice, can be attributed to placebo/contextual response, though the magnitude of this response may vary greatly between patients. We undertook this individual patient data (IPD) meta-analysis of three contrasting treatments for OA to identify placebo responders and the potential determinants of the placebo response in OA.

Methods: This study is undertaken in conjunction with the OA Trial Bank, an ongoing international consortium collecting IPD from randomised controlled trials (RCTs) for treatments of OA. Placebo-controlled RCTs for intra-articular (IA) corticosteroid injections, oral glucosamine tablets, and topical non-steroidal anti-inflammatory drugs (NSAID) have been systematically searched for and authors contacted to request the IPD. *Outcomes* The primary outcome measure for placebo response was maximum pain reduction over the duration of follow-up (1-119 weeks). Potential predictors and covariates available were intervention type, radiographic Kellgren and Lawrence (KL) score, sex, age, body mass index (BMI), duration of OA (years) and study joint. *Data Analysis* Pain was measured using different scales across trials and was normalised into a 0-100 scale for analysis. Maximum pain reduction was identified for each participant. Participants who achieved clinically important pain relief, defined as ≥20% reduction in pain score from baseline, were classified as responders. A one-stage IPD meta-analysis was used to analyse all studies simultaneously whilst accounting for heterogeneity across studies. A multilevel mixed-effects linear regression model was fitted. Maximum change from baseline pain score was the dependent variable, whereas baseline pain, age, sex, BMI and other potential predictors were independent variables. Univariate analysis was used to select significant predictors. Significant predictors were then examined in a multivariate model to confirm the results.

Results: *Characteristics of study population* Eighteen out of 19 identified trials provided IPD for this analysis. Data on 2,305 placebo participants were available for analysis. Of these studies, 4 trials (n=674 participants) used placebo tablets, 3 trials (n=78) used IA placebo injection and 11 trials (n=1,553) used topical placebo. Thirteen studies (n=1,764) included participants with knee OA, three (n=158) included participants with hip OA, and two (n=383) included participants with hand OA. *Placebo response* 75% of participants reported ≥20% pain reduction from baseline. The mean age was 61.94 years (95% CI 61.45 to 62.43) for responders and 61.85 (95% CI 61.00 to 62.70) for non-responders. The response rate in females was 75% and males 75%. The mean BMI was 30.05 (95% CI 29.73 to 30.38) for responders and 29.69 (95% CI 29.18 to 30.20) for non-responders. Absolute pain reduction with placebo was significant with an overall effect size of 25.97 (95% CI 20.39 to 31.55) on the 0-100 scale. *Predictors of placebo responders* In univariate analysis there was no difference in placebo response between men and women, nor according to age, route of delivery, or severity of structural OA (KL score). However, baseline pain, duration of OA symptoms and BMI were significantly associated with the overall change in pain score. In the subsequent multivariate model, these three predictors remained significant, with the effect of BMI being reversed (Table 1).

Table 1. Predictors of placebo response

		Univariate			Multivariate*	
	Number of studies	Number of patients	β (95% CI)	p	β (95% CI)	p
Baseline Pain (0-100)	18	2248	0.64 (0.59,0.69)	0.000	0.65 (0.58,071)	0.000
BMI (kg/m2)	16	2174	0.016 (0.01,0.31)	0.040	-0.26 (-0.47,-0.05)	0.018
Duration of OA (years)	9	772	-1.13 (-2.20,-0.06)	0.039	-0.98 (-1.88,-0.09)	0.031

*7 studies and 703 participants involved in multivariate analysis

Conclusions: This IPD meta-analysis demonstrates that people with higher baseline pain and shorter duration of symptoms may be more likely to respond placebo in OA but the direct effect of BMI remains uncertain. Interestingly, the route of delivery had no effect in contrast to the conclusions of previous studies. The findings of this study can be used to stratify study participants into subgroups based on their likely response to placebo and to improve the design of RCTs in order to develop a novel treatment that is truly better than placebo. Systematic review registration: PROSPERO CRD42016033212

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SIGNIFICANT PAIN REDUCTION WITH ORAL METHOTREXATE IN KNEE OSTEOARTHRITIS; RESULTS FROM THE PROMOTE RANDOMISED CONTROLLED PHASE III TRIAL OF TREATMENT EFFECTIVENESS

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Purpose: Current treatments for osteoarthritis (OA) are severely limited. Synovitis is prevalent in OA and is associated with pain. The slow-acting anti-rheumatic drug methotrexate (MTX) is the gold-standard treatment for synovitis in inflammatory arthritides. The primary aim of the PROMOTE trial was to determine the effectiveness of MTX versus placebo as an analgesic treatment for knee OA.

Methods: PROMOTE was a multi-centre, randomised, placebo-controlled trial with 12 months follow-up. Participants with symptomatic (visual analogue scale (VAS) pain ≥4/10) and radiographic tibiofemoral knee OA, fulfilling clinical ACR criteria, were recruited across UK primary and secondary care. Participants were randomized on a 1:1 basis to MTX or placebo, in addition to ongoing usual care, with dose escalation from 10mg to 25mg over 8 weeks and maintenance at 25mg (or the highest tolerated dose) for the remainder of the study. The primary endpoint was average knee pain during the previous week (numerical rating scale [0-10], NRS) at 6-months. Secondary endpoints included WOMAC, quality-of-life and adverse events. Linear mixed models compared outcomes between groups on an intention-to-treat (ITT) basis. A secondary complier average causal effect (CACE) analysis of the primary outcome at 6 months explored the treatment effect for patients who missed no more than 4 doses within any 3 months before the primary endpoint. In a sub-study, contrast-enhanced MRI of the index knee was performed at baseline and 6 months.

Results: Of 207 patients screened, 155 participants (64% women, mean age 60.9 years, 50% K-L Grade 3-4) were randomized. Primary endpoint data at 6 months were available for 134 patients (86%). At 6 months, average knee pain (as measured by NRS) was 6.2 in the placebo group and 5.1 in the MTX group, with a baseline adjusted treatment difference of -0.83 points (95% CI -1.55 to -0.10; p=0.025) in the primary ITT analysis, equivalent to a standard effect size of 0.36. Secondary adherence adjusted CACE analysis showed a treatment difference of -0.95 points (95% CI -1.72 to -0.18; p=0.019) (Table 1). Statistically significant differences at 6 months were seen for WOMAC stiffness and physical function, but not WOMAC pain (Table 2). Some treatment benefits were maintained to 9 months for NRS pain (p=0.075), WOMAC stiffness (p=0.016) and WOMAC physical function (p=0.071), but groups had comparable outcomes by 12 months. NSAID use was higher in the placebo group than the MTX group across all time points. 94 patients had analysable MRI data at baseline and 80 at 6 months; no change in synovial volume was found. Four serious adverse events were reported (MTX: 2 [not defined as possibly related], placebo: 2).

Conclusions: MTX added to usual care demonstrated significant reduction in knee OA pain at 6 months, and significant improvements in WOMAC stiffness and function. Despite a moderate standard effect size, the analgesic effect was of borderline clinical meaningfulness. Further analyses will explore predictors of response to understand if subsets with enhanced response can be identified. We acknowledge Versus Arthritis for funding support and the PROMOTE Investigators.