S100A9 seems less involved in peripheral sensitization in this acute synovitis. During the acute phase of inflammation S100A8/A9 is likely regulated via direct activation of TLR4 on nerve endings in the synovium and not via increased infiltration of phagocytes in the DRG. The finding that experimental OA (DMM) is partially mediated by TLR2 demonstrates the relevance of this finding for OA.

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IDENTIFICATION OF PUTATIVE SEX-DEPENDENT PAIN PATHWAYS THAT MAY EXPLAIN WHY FEMALE MICE DEVLOP PAIN BEHAVIOUR DESPITE LOWER CHONDROPATHY SCORES

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Purpose: The prevalence of osteoarthritis (OA) differs between sexes, whereby OA structural disease is substantially higher in females after the menopause. Pre-menopause, this trend is reversed. Females are also more likely to report pain and do so at lower structural disease scores. In murine models of OA it is established that young female mice show less chondropathy than male mice after joint destabilisation. Here we investigate whether there are differences in spontaneous pain behaviour in male and female mice and consider the molecular pain pathways that could be driving these sex-dependent differences.
Methods: Male and female C57BL6 and DBA1 mice were randomised to sham surgery or joint destabilisation by destabilisation of the medial meniscus (DMM) or partial meniscectomy (PMX). Additionally, a group of C57BL6 female mice, ovariectomized (OVX) at 6 weeks of age, underwent PMX surgery. Cartilage repair was assessed histologically 8 weeks following creation of a focal cartilage defect in the retro-patellar groove of male and female DBA1 mice. Spontaneous pain behaviour was assessed using Linton incapacitance testing. Laboratory Animal Behavior Observation Registration and Analysis System (LABORAS) was used to assess behavioural activity between sexes in naïve mice. Gene expression analysis was performed on whole knee joints 10 weeks post-PMX surgery. Disease severity was determined histologically from double blinded, modified Osteoarthritis Research Society International (OARSI) scores.
Results: In both strains and surgical models, females had reduced chondropathy scores compared with males following joint destabilisation. This did not appear to be due to reduced activity of female mice assessed by LABORAS, nor increased ability to repair. Despite reduced structural disease, both females and males displayed spontaneous pain behaviour starting at the same time post surgery (8 weeks following PMX, 10 weeks following DMM). No difference was observed in disease severity between OVX and non-OVX females nor in their pain behaviour. Molecular analysis of the joint at the time of pain behaviour showed regulation of NGF, Bdkrb2, Tacr1 and NPY in males in accordance with our previous published data. Some of these genes were also increased in females, albeit not reaching statistical significance. Females, on the other hand, showed significant upregulation of genes encoding glial derived neurotrophic factor (Gdnf), neurturin (Nrtn), neurotrophin 3 and 5 (Ntf3, Ntf5), and downregulation of persephin (Pspn).
Conclusions: Collectively these results confirm and extend previous reports showing that female mice have reduced chondropathy scores after joint destabilisation compared with males. Although, unlike previous studies we did not find that oophorectomy influenced this result. Despite reduced structural disease, female mice developed spontaneous pain-like behaviour at the same time as male mice suggesting that they have a lower threshold for pain. Molecular analysis of pain modulating genes uncovered regulation of a distinct set of pain sensitisers, only regulated in female joints at the time of pain development. Together this opens up the possibility that different molecular pathways are important for the onset and severity of painful behaviour in females compared with males. Such pathways may have clinical relevance as we start to develop personalised approaches in OA management.

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GENOME-WIDE ASSOCIATION STUDY OF WIDESPREAD PAIN LINKS LRRC3B AS POSSIBLE NOVEL PAIN ASSOCIATED GENE

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Purpose: The hallmark clinical symptom of clinical osteoarthritis (OA) is pain, which is one of the leading causes of disability in OA. Pain not only has a physical effect on the individual, but can also impact the individual mentally and socially. Despite this high burden, there is not a lot known on the role of genetics in musculoskeletal pain. In this study, we aimed to investigate the genetic determinants of muscoskeletal pain and the way we perceive it. In order to examine this, we have performed a genome-wide association study (GWAS) using the number of self-reported pain sites, as a proxy for musculoskeletal pain.
Methods: We have included 8,930 individuals (57.3% women) from the Rotterdam Study (RS) cohorts, RSI, RSII and RSII, with self-reported pain sites and genetic information available. Participants could indicate on 15 body sites if they were experiencing pain on these sites. Resulting in a score from 0-no pain at any site to 15-pain at every site. This score was used as outcome in the GWAS analysis. Genotype reference was imputed to HRC v1.1, GWAS analysis were adjusted for age and sex, in addition a sex stratified analysis was also performed. As the number of pain sites score did not have a normal distribution, top signals from the GWAS were additionally re-analyzed using a Poisson regression model. Top signals were followed up using gene expression information from GTEx database.
Results: The meta-analysis GWAS results of the Rotterdam study cohorts, did not show any genome-wide significant or suggestive associations ($p<1E^{-07}$) with number of pain sites. However the mean number of pain sites in women is 1.58 (SD=2.34), which is significantly higher than in men (0.84 SD=1.56, $p<1E^{-04}$). Thus we performed sex stratified analysis. In the female stratified GWAS one genome-wide significant signal was associated with number of pain sites, also after Poisson regression analysis (Beta= 0.50, $p_{poisson}=4.3*10^{-08}$) and was not associated with number of pain sites in men (beta=0.093, p=0.60). The lead variant rs1488240, with risk allele A has a MAF of 9% and is a novel pain associated variant. This variant is located near the Leucine-Rich Repeat Containing 3B (*LRRC3B*) gene and has a variant in high linkage disequilibrium which is a coding variant in *LRRC3B* ((rs35497952, r2 = 0.83, synonymous). GTEx expression data shows high expression of *LRRC3B* in multiple nervous system tissues. The strongest gene expression was reported in basal ganglia(n=194) and the cerebellum (n=209). No significant or suggestive signals were found in the male stratified analysis. In the female stratified analysis next to our one novel pain associated signal, we were also able to replicate 42 known pain associated variants($p<0.05$).
Conclusions: We have identified one novel pain associated variant, a synonymous variant in the *LRRC3B* gene, and nominally replicate 42 known pain associated variants. *LRRC3B* is highly expressed in the cerebellum and basal ganglia of the brain, which are both known to be involved in pain processing and modulation. In addition, *LRRC3B* has protein interactions and co-expression with *IGDCC3* which is linked to functions in axon guidance, and is highly expressed in mouse nervous systems and limb development. Suggesting *LRRC3B* might play a role in chronic musculoskeletal pain.

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ANATOMICAL DISTRIBUTION OF MRGPRD-EXPRESSING NONPEPTIDERGIC C-FIBERS IN THE MOUSE KNEE

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Purpose: The voltage-gated sodium channel, $Na_V1.8$, marks the majority of nociceptors, including more than 90% of C-fibers. We have used $Na_V1.8$ tdTomato reporter mice to describe the nociceptive innervation of the mouse knee. We reported that, 16 weeks after destabilization of medial meniscus (DMM), osteoarthritic (OA) joint damage is accompanied by extensive remodeling of nociceptors in the medial compartment of the knee, including increased $Na_V1.8+$ innervation in the medial synovium, in addition to the presence of $Na_V1.8+$ fibers in the medial meniscus and within subchondral bone channels. Distinct functional classes of C-fibers have been identified, where it has been proposed that TRPV1+ C-fibers mediate heat sensitivity and C-fibers that express the G protein-coupled receptor (GPCR), Mrgprd (The Mas-related G protein-coupled receptor D), mediate behavioral sensitivity to noxious mechanical stimuli. Mrgprd marks about 75% of all $IB4^+$ nonpeptidergic nociceptive neurons and are believed to innervate the skin, while IB4+ C-fibers have been reported to be absent in rat joints. Their potential role in mechanosensation makes Mrgprd expressing neurons an interesting subject in the context of OA pain,