Short Report

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Single nucleotide polymorphism in the *COL11A2* gene associated with lowered heat pain sensitivity in knee osteoarthritis

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Abstract

Pain is one of the most prominent symptoms of osteoarthritis. However, there is often discordance between the pain experienced by individuals with osteoarthritis and the degree of articular pathology. This suggests that individual differences, including genetic variability in the central processing of nociceptive stimuli, may impact the presentation of osteoarthritis. Here, we show that the single nucleotide polymorphism rs16868943 in the collagen gene *COL11A2* is significantly associated with lowered heat pain tolerance on the arm in participants with knee osteoarthritis ($P = 1.21 \times 10^{-6}$, $P = 0.0053$ after Bonferroni correction, beta $= -3.42$). A total of 161 knee osteoarthritis participants were included and evaluated for heat, punctate and pressure pain sensitivity of the affected knee and the ipsilateral arm. Each participant was genotyped for 4392 single nucleotide polymorphisms in genes implicated in pain perception, inflammation and mood and tested for association with pain sensitivity. The minor A allele of single nucleotide polymorphism rs16868943 was significantly associated with lower arm heat pain tolerance after correction for age, gender, race, and study site. This single nucleotide polymorphism was also nominally associated with other measures of heat pain sensitivity, including lowered knee heat pain tolerance ($P = 1.14 \times 10^{-5}$, $P = 0.05$ after Bonferroni correction), lowered arm heat pain threshold ($P = 0.0039$, uncorrected) and lowered knee heat pain threshold ($P = 0.003$, uncorrected). Addition of genotypes from 91 participants without knee pain produced a significant interaction between knee osteoarthritis status and the rs16868943 single nucleotide polymorphism in heat pain tolerance ($P = 1.71 \times 10^{-5}$), such that rs16868943 was not associated with heat pain tolerance in participants without knee pain ($P = 0.12$, beta $= 1.3$). This is the first study to show genetic association with heat pain tolerance in individuals with osteoarthritis. The association is specific to participants who have already developed knee osteoarthritis, suggesting that the *COL11A2* gene, which has previously been associated with familial osteoarthritis, may play a role in pain sensitization after the development of osteoarthritis.

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Keywords
Osteoarthritis, knee osteoarthritis, pain genetics, association study, pain sensitivity, allodynia, *COL11A2*, heat pain, pain tolerance

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Introduction

Pain is the most common symptom of osteoarthritis (OA), yet the determinants of OA-related pain remain poorly understood. Objective measures of OA severity are at best only modestly associated with pain severity, suggesting that individual differences in pain processing may contribute to OA-related pain. Indeed, heightened experimental pain sensitivity has been observed in participants with knee OA, and experimental pain sensitivity predicts severity of clinical pain in these participants.[1–6] Understanding the factors driving heightened pain sensitivity in knee OA could provide clinically relevant information.

The experience of pain is a complex process determined by multiple biological and environmental factors, including genetic influences. The genetic contribution to pain sensitivity has been estimated to be 22–55% in twin studies.[7] Heritability for nociceptive and analgesic sensitivity in mice similarly ranges from 28% to 76%.[8] Several genes have been implicated in increased pain sensitivity in humans. The most-studied gene in pain research is the catechol-O-methyl-transferase (*COMT*) gene. *COMT* polymorphisms and haplotypes have been associated with both experimental pain sensitivity and clinical pain phenotypes. Several other genes have likewise shown associations with experimental pain sensitivity in multiple studies, including *OPRM1*,[9] *GCH1*,[10,11] *MC1R*,[12] and *SCN9A*[13,14] and dopamine transporter genes (*SLC6A3* and *SLC6A4*).[15]

In OA-related pain, several genes have been associated with clinical pain severity in candidate gene studies. For example, the Val158Met polymorphism in the *COMT* gene (rs4680) was associated with increased hip OA pain,[16] though a subsequent study failed to observe any association of this single nucleotide polymorphism (SNP) with knee OA.[17] Also, SNP rs900414 in the *PCSK6* gene was associated with protection against pain when radiographic OA was present.[18]

Despite these recent advances in our knowledge of genetic contributions to OA, our understanding of genetic factors associated with pain sensitivity in OA remains limited. Previous studies have focused on a few very specific variants in candidate genes, making it difficult to discover potential disease-associated variants outside of the hypotheses. The primary aim of this study is to significantly expand the tested genes and polymorphisms, with the hope to discover new risk genes and alleles for pain sensitivity in OA. To address this aim, we performed an association study including 4392 SNPs in 555 genes implicated in pain, inflammation, and psychiatric disorders. We performed extensive pain phenotyping, including assessment of clinical pain and quantitative sensory testing to assess sensitivity to heat, cold, pressure, and punctate pain. To characterize pain sensitivity at sites remote from the primary painful joint in OA participant, pain sensitivities were assessed not only on the most painful knee (i.e., index knee) but also at body sites outside the index knee.

Material and methods

Study subjects

Recruitment of knee OA participants in the study has been described elsewhere.[19] Briefly, 45- to 85-year-old individuals, who self-identified their race/ethnicity as African American or Non-Hispanic Whites, were enrolled in the study at the University of Florida and University of Alabama at Birmingham. Participants were recruited who screened positive for unilateral or bilateral symptomatic knee OA[20] and physical examination subsequently confirmed knee pain symptoms consistent with a clinical diagnosis of knee OA, regardless of radiographic evidence. At the time of study entry, posteroanterior and lateral radiographs of the knees were obtained from all participants, with the knees in a bilateral weight-bearing, fixed-flexion position, as described elsewhere.[21]

Participants were excluded if any of the following features were present: (1) prosthetic knee replacement or other clinically significant surgery to the affected knee; (2) uncontrolled hypertension (blood pressure >150/95 mm Hg), heart failure, or history of acute myocardial infarction; (3) peripheral neuropathy; (4) systemic rheumatic disorders including rheumatoid arthritis, systemic lupus erythematosus, and fibromyalgia; (5) daily opioid use; (6) cognitive impairment; or (7) hospitalization for psychiatric illness within the preceding year. Individuals with no knee pain (i.e., controls) met the same inclusion/exclusion criteria, with the exception that they reported no knee pain.

A total of 177 participants with knee OA and 99 participants without knee pain were genotyped. Participants with missing gender, age, race, or study sites were excluded from the analysis. Participants with greater than 10% missing genotype were also excluded. After exclusion criteria were applied, 161 knee OA and 91 participants without knee pain were included in the analysis.

Pain measurement

Pain measurements have been described elsewhere.[19] The index knee here is defined as the most painful knee.

Heat. Heat–pain threshold and tolerance levels were assessed on both the index knee and the ipsilateral ventral forearm, starting at the baseline temperature (32°C) and increasing 0.5°C/s. Participants were instructed to press a button when the sensation "first became painful" (i.e., threshold) and also when they "no longer felt able to tolerate the pain" (i.e., tolerance). The mean temperature from three trials was used for analysis.

Pressure. Pressure–pain thresholds were evaluated at the medial joint line of the index knee as well as the ipsilateral trapezius. For each site, a handheld Medoc digital pressure Algometer (Algomed) was applied at a constant rate of 30 kPa/s. The participant was instructed to press a button when the sensation "first became painful." An average pressure–pain threshold was determined for each site from three trials. The maximum pressure for the knee site was 600 and 1000 kPa at the ipsilateral trapeziius. If participants did not report pain at the maximum pressure level, the procedure was terminated and a pressure of 600 or 1000 kPa was assigned for that trial.

Punctate. Sensitivity to punctate mechanical stimuli was assessed at the index patella and back of the ipsilateral hand using a nylon monofilament delivering a target force of 300 g. Participants provided verbal pain ratings following a single contact and after 10 contacts, at a rate of 1 contact/s. Ratings were made on a scale of 0 to 100. The procedure was repeated, and the ratings for multiple contacts were averaged for analysis.

Cold sensitivity. Participants placed their right hand into a 12°C cold water bath for up to 1 min. Participants provided verbal ratings of pain intensity at the end of the immersion on a scale of 0 to 100. If a participant withdrew the hand from the water bath before the 1-min period ended, the pain rating was collected and the withdrawal time was recorded.

Graded Chronic Pain Scale. The Graded Chronic Pain Scale (GCPS) evaluates global pain severity and pain-related interference over the past six months and consists of seven items related to pain intensity and pain interference.[22] With a 0–10 numeric rating scale, participants rated the intensity of their current knee pain and the worst and average pain during the past six months. These three items were averaged and multiplied by 10 to generate a GCPS characteristic pain intensity score.

Western Ontario and McMaster Universities Osteoarthritis Index. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[23] assesses symptoms of knee OA in the past 48 h. For this study, the 4-point Likert-type scale version was used. The WOMAC yields three subscales, including pain during activities (5 items), stiffness during the day (2 items), and impairments in physical function (17 items), with higher scores indicating worse pain, stiffness, and impairments in physical function.

Genotyping

Knee OA participants and healthy participants were genotyped in the same fashion. At enrollment, whole blood was collected by venipuncture from study participants who provided consent for genotyping. Blood was collected into 5-mL ethylenediamine tetra-acetic acid–containing Vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ), and leukocytes were purified and stored at −80°C. Genomic DNA was purified utilizing protocols based on PureGene Extraction Kits from Qiagen (Germantown, MD). All individuals were genotyped using the Algynomics (Chapel Hill, NC) Pain Research Panel, a dedicated chip-based platform manufactured by Illumina. The panel assesses 4900 SNPs representing 555 genes known to be involved in systems relevant to pain perception (complete list provided in the Supplementary Material 1). DNA sample concentrations were measured with PicoGreen (ThermoFisher Scientific, Waltham, MA) and diluted to 50 ng/μl in 96-well plates. These were genotyped using the Algynomics chips, run on an Illumina VeraCode instrument (Illumina, San Diego, CA). SNPs were excluded when minor allele frequency was 1% or less, Hardy–Weinberg equilibrium $P < 10^{-5}$, and SNP call rate 95% or less. After quality control, a total of 4392 SNPs were included in the analysis.

Statistical analysis

Associations between each phenotype and each individual SNP were tested with PLINK using the additive genetic model in linear regression,[24] with age, sex, study sites, and race as covariates. A similar analysis including weight as an additional covariate was applied to the significantly associated SNP. Bonferroni correction for testing 4392 SNPs was applied to the adjusted P value. The study-wide Bonferroni-corrected statistical significance level was set at $P = 1.14 \times 10^{-5}$. A subanalysis was carried out in Africans Americans and Europeans Americans for heat pain tolerance and threshold. The rs16868943 polymorphism, being the only significant SNP associated with the arm heat tolerance phenotype after Bonferroni correction, was selected for further analysis in participants without knee pain. In this population, a similar association study was carried out between rs16868943 and arm heat tolerance. A

Table 1. Demographics and pain sensitivity of knee OA and healthy participants.

Phenotype	Knee OA (N = 161)	No knee pain (N = 91)	*P*
Age	56.83	56.81	0.99
Female (%)	73	68	0.34
African American (%)	51	25	0.000067
Annual Income (10k)	4.25	4.75	0.21
Heat pain threshold, ipsilateral arm (°C)	41.5	42.74	0.0017
Heat pain threshold, index knee (°C)	41.64	42.23	0.16
Heat pain tolerance, ipsilateral arm (°C)	45.92	46.87	0.0017
Heat pain tolerance, index knee (°C)	45.68	46.68	0.0013
Pressure pain threshold, index knee (kPa)	288.41	369.04	0.00014
Pressure pain threshold, ipsilateral trapezius(kPa)	271.54	350.24	0.0015
Punctate pain, index knee (out of 100)	34.68	23.81	0.0032
Punctate pain, ipsilateral hand (out of 100)	25.33	14.61	0.00034

Participants with knee OA in general have greater pain sensitivity compared to participants without knee pain. *P* value is calculated from two-tailed student's *t*-test.
OA: osteoarthritis.

subanalysis was carried out in Africans and European Americans. The interaction between the heat pain tolerance in the arm and knee OA phenotype was also tested. Interaction P value was calculated using the linear regression model in PLINK, controlling for age, sex, study sites, and race. The locus plot was generated by LocusZoom.[25] Comparisons of pain measures between knee OA and participants without knee pain were calculated with two-tailed Student's *t*-test.

Results

A total of 161 participants with knee OA and 4392 SNPs in 555 genes were included in the final analysis after quality control. The average age of the participants was 56.8 years, with 73% female and 27% male, 49% European Americans, and 51% African Americans. Ninety-one participants without knee pain were also included for comparison, with an average age of 56.8 years, 32% male and 68% female, 75% European Americans, and 25% African Americans. Participants with knee OA, compared to participants without knee pain, in general had elevated sensitivity to heat, pressure, and punctate pain in both the index knee and ipsilateral arm (Table 1). This is consistent with previous findings from this cohort and others showing that participants with knee OA have generalized pain sensitization.[23,26–29] Association studies were carried out between SNPs and clinical as well as experimental pain measures with linear regression, adjusting for age, gender, race, and study sites. The top associated SNPs for each pain measure are listed in Table 2. Based on the study-wide Bonferroni-corrected statistical significance level of $P = 1.14 \times 10^{-5}$, the only SNP that reached statistical significance for association with any pain measure was rs16868943 in the *COL11A2* gene, which was found to be associated with lower heat pain tolerance in the ipsilateral arm at $P = 1.21 \times 10^{-6}$ ($P = 0.0053$ after Bonferroni correction, and beta = −3.42). Because weight is a risk factor for knee OA pain, we added weight as an additional covariate for comparison but it did not significantly alter the association P value ($P = 1.08 \times 10^{-6}$). The average heat pain tolerance in knee OA participants with the GG genotype is 46.18°C, but the tolerance for participants with the GA genotype is 42.08°C (Figure 1(a)). There was no AA genotype present in our data set, likely secondary to the low minor allele frequency. This SNP was also marginally associated with lower heat pain tolerance in the index knee ($P = 1.14 \times 10^{-5}$, corrected $P = 0.05$, beta = −3.67), lower heat pain threshold in the ipsilateral arm ($P = 0.0039$, corrected $P = 1$, beta = −2.83) and knee ($P = 0.0030$, corrected $P = 1$, beta = −2.9), higher punctate pain sensitivity in the ipsilateral hand ($P = 0.0065$, corrected $P = 1$, beta = 22) and higher cold pain sensitivity ($P = 0.049$, corrected $P = 1$, beta = 21.58). It was not associated with punctate pain in the index knee ($P = 0.30$), pressure pain of the index knee ($P = 0.39$), or ipsilateral trapezius ($P = 0.32$), WOMAC score ($P = 0.33$), or the GCPS score ($P = 0.77$) (Table 2).

Because the minor allele frequency (A allele) of this SNP is higher in African Americans than European Americans, associations with heat pain tolerance/threshold were examined separately by race. Knee OA participants with the GA genotype, compared to the GG genotype, had lower heat pain tolerance/threshold in the ipsilateral arm and ipsilateral leg within both African Americans and European Americans (Figure 1

Table 2. Top SNPs associated with each pain measure.

SNP	Gene	Functional consequence	Chr	Position	Major allele	Minor allele	Minor allele frequency	Beta	*P* (uncorrected)	*P* (Bonferroni corrected)
Heat pain tolerance, ipsilateral arm										
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−3.42	1.21×10^{-6}[a]	0.0053[a]
rs11129802	SCN10A	Intron variant	3	38750436	C	T	0.17	1.06	0.00024	1
Heat pain tolerance, index knee										
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−3.67	1.14×10^{-5}	0.05
Heat pain threshold, ipsilateral arm										
rs2020933	SLC6A4	Intron variant	17	28561755	A	T	0.15	1.59	0.0004	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−2.83	0.0039	1
Heat pain threshold, index knee										
rs7103411	BDNF-AS, BDNF	Intron variant	11	27700125	T	C	0.25	2.29	0.0001	0.44
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−2.90	0.0030	1
Punctate-induced pain, ipsilateral hand										
rs12415832	GRK5	Intron variant	10	121112327	C	A	0.055	25.02	0.00046	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	22.00	0.0065	1
Punctate-induced pain, index knee										
rs2398144	GNAO1	Intron variant	16	56352854	A	C	0.43	−9.94	0.00051	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	8.78	0.30	1
Pressure-induced pain threshold, index knee										
rs3790112	GNAO1	Intron variant	16	56381197	G	T	0.38	−61.51	0.00013	0.57
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−41.10	0.39	1
Pressure-induced pain threshold, ipsilateral trapezius										
rs1059829	SPARC	utr variant 3 prime	5	151042029	G	A	0.39	65.96	0.00013	0.57
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−52.64	0.32	1
Cold sensitivity										
rs1479922	IL2	Intergenic	4	123371785	G	A	0.016	36.05	0.00071	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	21.58	0.049	1
Graded chronic pain scale										
rs6812524	SPP1	Synonymous codon	4	88902725	G	A	0.073	12.15	0.00072	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	2.10	0.77	1
Western Ontario and McMaster Universities Osteoarthritis Index										
rs17586428	HTR2B, PSMD1	Intron variant	2	231988855	A	G	0.18	2.20	0.00033	1
rs16868943	COL11A2	Intron variant	6	33147727	G	A	0.034	−1.34	0.33	1

The study-wide significant *P* value is set at 1.14×10^{-5} after Bonferroni correction for 4392 SNPs included in the study.

SNP: single nucleotide polymorphism.

[a]rs16868943 is associated with decreased heat pain tolerance after Bonferroni correction. Associations of this SNP with each pain measure are included in this table.

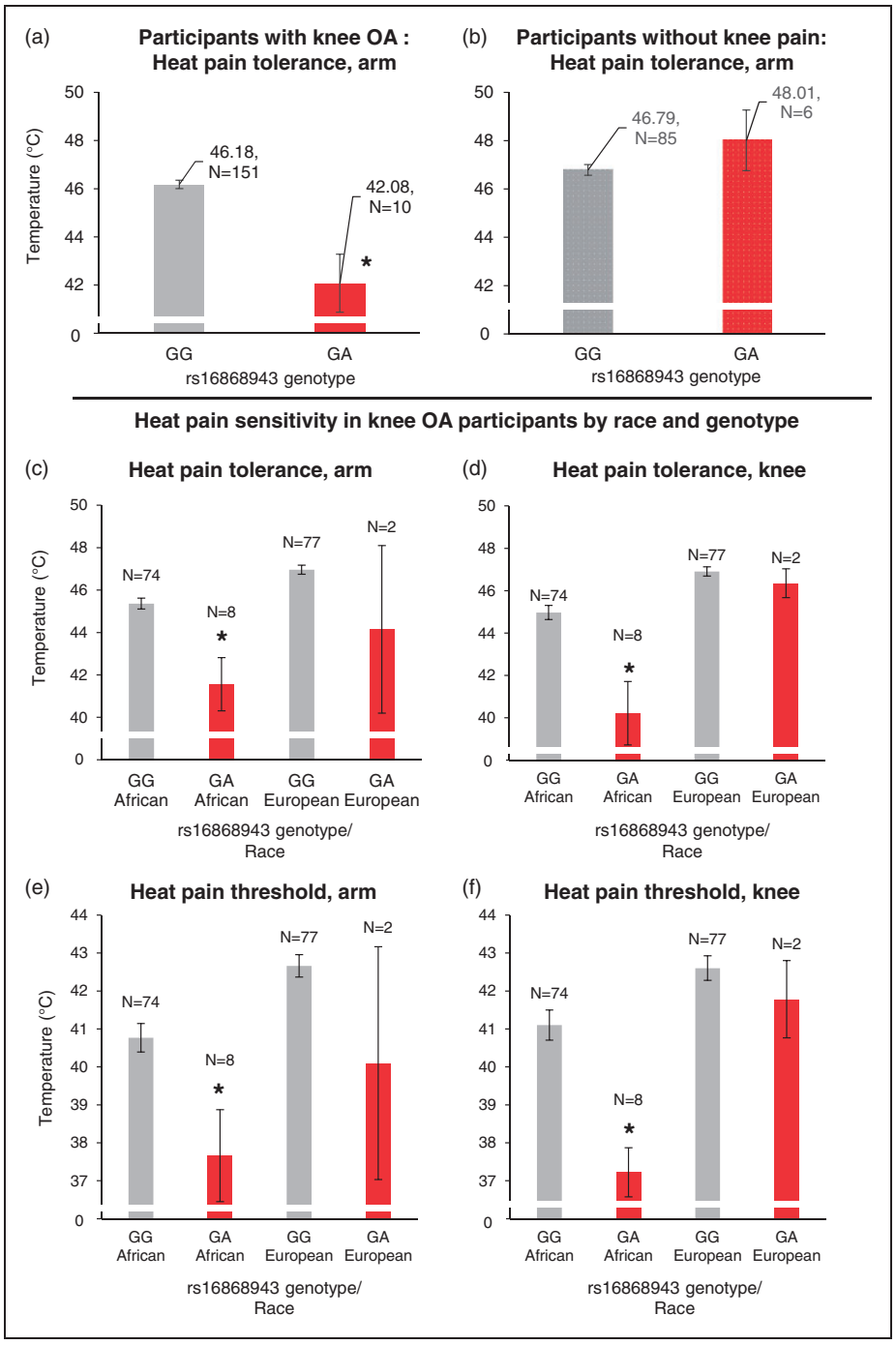

Figure 1. Heat pain sensitivity by phenotype and genotype. (a) The s16868943 polymorphism is associated with decreased arm heat pain tolerance in participants with knee OA ($P = 1.21 \times 10^{-6}$, $P = 0.0053$ after Bonferroni correction, beta $= -3.42$), but not in participants without knee pain; (b) the heat pain tolerance and threshold are broken down by race and genotype in (c–f). The GA genotype is nominally associated with lower heat pain tolerance and threshold in the index knee in African Americans but not in European Americans.
*Statistically significant association of s16868943 with the specific phenotype (uncorrected $P < 0.05$). For a complete listing of uncorrected and corrected P values, please refer to the Results section.

(c)—(f). Within African Americans, the presence of the A allele was associated with lower heat pain tolerance in the index knee ($P = 4.91 \times 10^{-5}$, corrected $P = 0.21$, beta $= -4.68$) and the ipsilateral arm ($P = 3.21 \times 10^{-5}$, corrected $P = 0.14$, beta $= -3.74$), and lower heat pain threshold in the index knee ($P = 0.0037$, corrected $P = 1$, beta $= -3.59$) and ipsilateral arm ($P = 0.0018$, corrected $P = 1$, beta $= -2.90$), compared to the G allele.

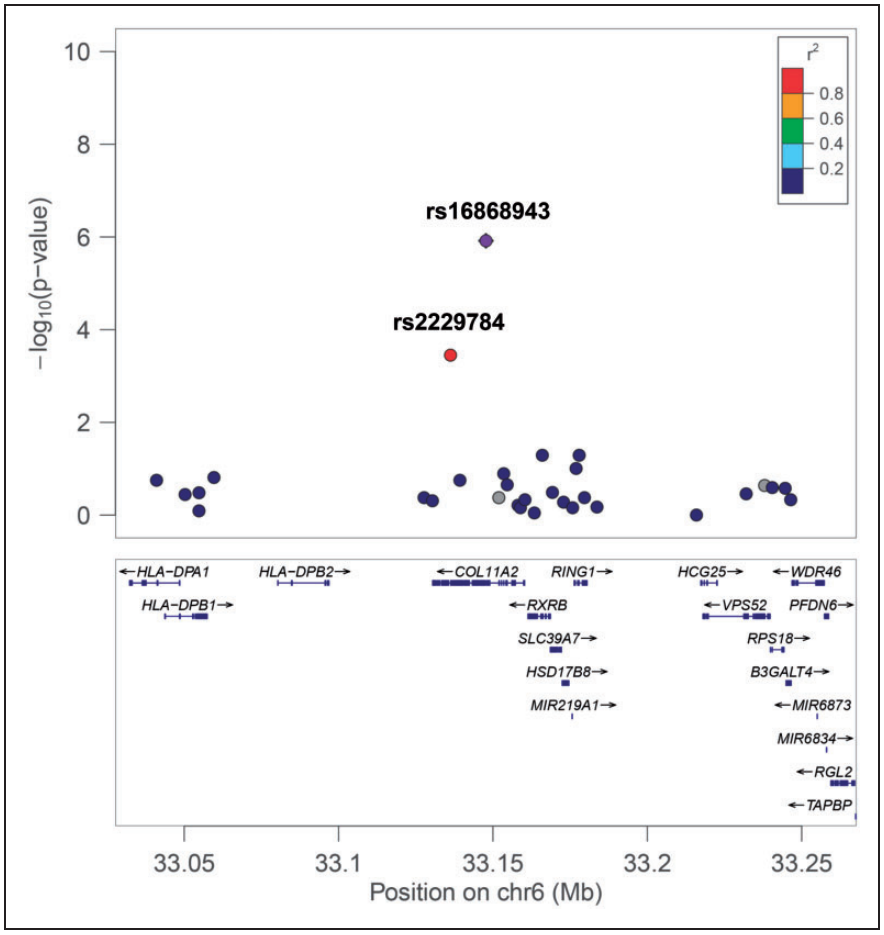

Figure 2. Genetic structure around the s16868943 polymorphism. The rs16868943 polymorphism lies in the *COL11A2* gene. The rs2229784 polymorphism is a missense mutation (Pro → Thr) in the *COL11A2* gene in strong linkage disequilibrium with rs16868943.

Within European Americans, the A allele was not statistically associated with heat pain tolerance in the index knee ($P = 0.91$, beta $= -0.14$), the ipsilateral arm ($P = 0.072$, beta $= -2.38$), or heat pain threshold in the index knee ($P = 0.77$, beta $= -2.57$) and ipsilateral arm ($P = 0.25$, beta $= -2.09$). The P value approached significance for the ipsilateral arm in European Americans, but there was reduced power due to the lower A allele frequency in this population. The direction of the association was the same between European and African Americans, with presence of the A allele related to reduced pain tolerance.

We carried out a follow-up association study between rs16868943 and participants without knee pain, to test whether this polymorphism also is associated with decreased heat pain tolerance in participants without knee pain. The result is shown in Figure 1(b). There was no association found between arm heat pain tolerance and rs16868943 in participants without knee pain ($P = 0.12$). The data trend was also opposite of that seen in participants with knee OA (beta $= 1.3$). A subanalysis was carried out in African Americans and European Americans without knee pain (Supplementary Material 2, Figure 1). In African Americans, there was no association between arm heat pain tolerance and the minor allele of rs16868943 ($P = 0.31$, beta $= -0.85$). In European Americans, there was a significant association ($P = 0.03$), but the trend was opposite of that seen in participants with knee OA (beta $= 2.68$). Because of the discrepancy seen between participants with knee OA and participants without knee pain, we asked whether the genotypes of rs16868943 interact with the presence of knee OA. After controlling for race, gender, age, and study site, a significant interaction was found between the minor allele of rs16868943 and the presence of knee OA ($P = 1.71 \times 10^{-5}$, see Supplementary Material 2, Figure 2 for illustration.). This result suggests that the effect of this polymorphism is dependent upon the development of knee OA. Its effect on pain perception is not present in participants without knee pain.

The genomic structure and P values of SNPs surrounding rs16868943 are shown in Figure 2. The

rs16868943 polymorphism is in high linkage disequilibrium (LD) with many local SNPs, but only rs2229784 is included in the study. The rs2229784 polymorphism is a missense mutation (Pro → Thr) variant within the *COL11A2* gene. It is associated with lower arm heat pain tolerance at $P = 0.00035$ (corrected $P = 1$). This finding implies that the rs16868943 polymorphism, an intron variant in the *COL11A2* gene, and the rs2229784 polymorphism, a missense variant in the *COL11A2* gene may both play a role in the development of increased pain sensitivity, or that other genetic variants within the haplotype are conferring or contributing to the effect.

Discussion

Pain sensitivity is influenced by multiple individual difference factors, including genetic variability. We hypothesize that natural genetic variations within pain-related genes affect the development of such hypersensitivity in participants with knee OA. Here, we showed that the minor (A) allele of rs16868943 in *COL11A2* is significantly associated with lowered arm heat tolerance in participants with knee OA. This SNP is also nominally associated with other measures of heat pain hypersensitivity, including lowered knee heat pain tolerance, lowered arm heat pain threshold, and lowered knee heat pain threshold. Since minor allele frequency is higher in the African American population (7%) compared to the non-Hispanic Whites (1.4%), a race-specific analysis was carried out. The same finding remains significant in African Americans and the trend is similar within non-Hispanic Whites. Furthermore, an association study carried out between rs16868943 and arm heat pain tolerance was found to be non-significant in participants without knee pain.

The rs16868943 SNP is located within an intron of the *COL11A2* gene, coding for the protein Collagen Type XI Alpha 2 Chain. This SNP is scored a 5 (TF binding or DNase peak) on RegulomeDB,[30] with unclear functional implication. However, this SNP is in high LD with several SNPs with high regulatory potentials. Over 20 SNPs are in high LD ($R^2 > 0.7$), with these SNPs in the European American and African American population (for details, please see LDProxy using the ASW and CEU population on the LDlink website[31]). These SNPs may represent potential causative SNPs. Most of these SNPs are within the *COL11A2* gene, including the missense variant rs2229784, but some of these SNPs are within other nearby genes, including *HLA-DPA1*, *HLA-DPB1*, *HLA-DPB2*, and *RING1*. Therefore, rs16868943 may simply be a proxy for other causative, genetically linked polymorphisms. The A allele of rs16868943 has also been associated with Celiac disease in a GWAS study[32] at a genome-wide significant level ($P = 2.06 \times 10^{-11}$). The clinical significance of such an association is unclear.

The *COL11A2* gene has been linked to the development of OA in several studies. A splicing mutation in the gene has been associated with early onset OA.[33] Mutations in *COL11A2* have also been associated with Stickler's syndrome,[34,35] a syndrome commonly accompanied with joint pain, stiffness, and inflammation. It is also the disease gene for the autosomal-dominant and recessive forms of osteochondrodysplasia.[36] In animal model, the *COL11A2* knock-out mouse shows an increased rate of developing knee OA.[37] *COL11A2* has not been associated with OA in several recent GWAS studies,[38–40] but its sister gene, *COL11A1*, has been associated with OA in a meta-analysis of nine genome-wide association studies.[41] Despite these findings, no study to date has suggested the role of *COL11A2* in pain perception among individuals with OA. This is the first study to suggest that the A allele of rs16868943, within *COL11A2,* is associated with increased heat pain sensitivity in participants with knee OA. This finding expands the potential influence of the *COL11A2* gene to not just the development of OA but potentially to the development of the widespread painful sensation in participants with OA.

The minor allele frequency of rs16868943 differs among racial populations, being higher in the African American population (7%) than the European Americans (1.4%). African Americans in general have enhanced sensitivity to noxious stimuli, including lower heat pain tolerance compared to their European American counterparts.[19,42–44] The higher allele frequency of this polymorphism in African Americans may partially contribute to their lower pain tolerance compared to European Americans. Despite the higher allele frequency, our analysis showed that African Americans still displayed higher pain sensitivity and clinical pain levels after controlling for the rs16868943 genotype. This finding suggests that there are factors other than the rs16868943 genotype causing the lower pain tolerance within African Americans, whether it be other genetic or environmental factors.

The mechanism by which *COL11A2* gene produces heightened heat pain sensitivity in people with knee OA is unclear. One possibility is that *COL11A2* is related to the pathology of OA and heightened pain sensitivity is the indirect consequence of the chronic OA disease. However, multiple GWAS studies have failed to show an association of *COL11A2* with OA, which argues against *COL11A2* as a causative OA gene. Perhaps the effect of *COL11A2* is somehow activated by the genetic predispositions of those who developed knee OA. This is consistent with the significant interaction in heat pain

sensitivity between the presence of knee OA and the genotype of rs16868943, suggesting that this polymorphism does not play a role in heat pain until knee OA has developed. Given that *COL11A1*, a sister gene of *COL11A2*, has been found to be associated with OA in a recent meta-analysis, one possible genetic explanation is that *COL11A1* and *COL11A2* may be working in concert in increasing heat pain sensitivity. Unfortunately, *COL11A1* was not included in our study so this hypothesis could not be addressed. Another interesting observation is that *COL11A2* solely affects heat pain sensitivity and thresholds but not other clinical or experimental pain measures. The reason behind such a difference is again unclear. *COL11A2* may be only involved in the transduction pathway of heat pain[45] but not pathways involving cold, punctuate, or pressure pain.

Central pain sensitization has been hypothesized as one of the mechanisms producing pain in individuals with OA. Quantitative sensory studies indicated that OA individuals are more sensitive to experimental pain stimuli than healthy individuals, particularly among individuals with high levels of clinical pain.[26–29] Notably, the increased pain sensitivity is not only present at sites close to the affected knee[29] but also at sites remote from the primary painful joint, including the forehead[46] and the arms.[29,47] Within our study, the rs16868943 polymorphism of *COL11A2* conferred decreased heat pain tolerance and threshold in both the ipsilateral arm and knee. This indicates that the effect of *COL11A2* on heat pain responses is widespread and not just limited to the ipsilateral knee. Such an observation suggests the possible role of *COL11A2* in producing wide-spread pain sensitization in knee OA.

There are several limitations to this study. First, the findings are limited to the genes and SNPs included in this study. Second, all the experimental tasks were acute, controlled painful experiences. Given the artificial nature of the experimental procedures, the outcomes may have limited practical utility. However, several studies have shown the relevance of using experimental pain induction procedures to predict clinical pain[1–6] In addition, our sample size was relatively small for a candidate gene study, especially given the low minor allele frequency of rs16868943 in non-Hispanic whites. Thus, replication of these findings in a larger sample is needed. Another limitation is that because it is a retrospective study, it is difficult to know whether the participants developed higher pain sensitivities after developing knee OA. A prospective study is needed to more confidently conclude that the rs16868943 A allele, or related haplotype, is associated with increased pain sensitivity before and after onset of knee OA. Because this study was initially designed to examine ethnic differences, roughly half of the participants were African American and half were European Americans. Although self-reported ethnicity was controlled in the linear regression analysis, underlying population stratification may have confounded the outcome.

These limitations notwithstanding, our findings indicate that the rs16868943 polymorphism in the *COL11A2* gene may be a marker for increased heat pain sensitivity in participants with knee OA. Additional research is warranted to replicate and expand this finding.

Declaration of Conflicting Interests

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