BRIEF REPORT

Role of the Na$_V$1.7 R1150W Amino Acid Change in Susceptibility to Symptomatic Knee Osteoarthritis and Multiple Regional Pain

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Objective. To assess the genetic association of pain in patients with knee osteoarthritis (OA) and those with multiple regional pain with the R1150W variant in the α-subunit of the voltage-gated sodium channel Na$_V$1.7.
Methods. Knee OA patients from 2 UK cohorts (1,411 from the Genetics of Osteoarthritis and Lifestyle study and 267 from the Hertfordshire Cohort Study; 74% with symptomatic OA) with Western Ontario and McMaster Universities OA Index (WOMAC) pain scores were genotyped for rs6746030 (encoding the R1150W change). One hundred seventy-six knee OA patients (53% symptomatic) from the Clearwater Osteoarthritis Study were also tested. A total of 4,295 samples (both affected and unaffected OA) from all 3 studies with data on multiple regional pain were tested. Fixed-effects meta-analyses were carried out with the WOMAC, symptomatic OA (adjusting for radiographic severity), and multiple regional pain as outcomes.
Results. No association with the WOMAC was seen in the UK cohorts. Overall, the meta-analysis of WOMAC yielded a summary statistic of $\beta = 0.47$ (95% confidence interval [95% CI] 0.04, 0.89; $P = 0.030$) for the variant allele. The meta-analysis of symptomatic versus asymptomatic OA did not demonstrate an association with rs6746030 (odds ratio [OR] 0.90 [95% CI 0.71, 1.15], $P = 0.38$). The meta-analysis of multiple regional pain resulted in a significant OR of 1.40 (95% CI 1.08, 1.80; $P = 0.0085$). No interstudy heterogeneity was seen for any of the analyses.
Conclusion. We find evidence that the R1150W amino acid change in the Na$_V$1.7 α-chain is associated with multiple regional pain. This variant is confirmed to be involved in genetic susceptibility to pain, but it does not appear to have a major role in OA-specific pain.

Introduction

The American College of Rheumatology has highlighted that pain is the main symptom of patients with rheumatic disorders (1). Chronic pain is common, challenging, and costly, affecting approximately 15% of the population, increasing the consultation rate 5-fold, and representing £18 billion in lost earnings in the UK in 2002 (2). Relief

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from severe chronic osteoarthritis (OA) pain remains a major reason for seeking surgical intervention and constitutes an unmet medical need (3). Therefore, understanding the molecular pathogenesis of chronic pain and specifically OA-related pain is of clinical and socioeconomic importance.

The only study published to date investigating genetic factors that contribute to painful knee OA has implicated the *SCN9A* gene, which encodes the α-subunit of the tetrodotoxin-sensitive voltage-gated sodium channel $Na_V1.7$ (4). Tissue damage depolarizes sensory neurons, but the transmission of information to the central nervous system (CNS) requires the recruitment of ion channels to propagate action potentials and cause neurotransmitter release into the CNS. One such channel present on peripheral nerves is $Na_V1.7$. A mutation that leads to loss of function of this channel is associated with insensitivity to pain, whereas mutations causing increased function lead to erythromelalgia or paroxysmal extreme pain disorder (see the references list in ref. 4). Recently, Reimann and colleagues (4) tested 27 single-nucleotide polymorphisms (SNPs) in the *SCN9A* gene and found an SNP associated with higher (worse) Western Ontario and McMaster Universities OA Index (WOMAC) pain scores in 578 large joint OA cases. The G>A SNP rs6746030 encodes an R1150W amino acid change, and the variant allele A (corresponding to the amino acid W) was also significantly associated with sciatica pain and postamputation pain. A similar but not significant trend was seen with this variant in postdiscectomy pain. The amino acid change was found to correspond to a statistically significant difference in the voltage-dependent slow activation where the variant A (W) allele would be predicted to increase $Na_V1.7$ activity.

The report by Reimann and colleagues, however, did not attempt replication in additional OA cohorts (4). Replication and subsequent meta-analysis of genetic results from different cohorts provide a quantitative approach for combining the results of various studies to estimate the true genetic risk conferred by any variant in human disease (5). Our aim was to assess whether the variant allele in the *SCN9A* gene involved in increased $Na_V1.7$ activity is indeed associated with higher WOMAC scores in people with knee OA. In addition, we explored the role of this variant in symptomatic versus asymptomatic cases and in more generalized pain phenotypes such as multiple regional pain taking advantage of 3 extant collections with knee OA and pain data.

Subjects and Methods

Three study cohorts, 2 from the UK and 1 from the US, were used. Assembly of the cohorts was approved by the local research ethics committees and all of the study participants gave fully informed consent to participate in genetic studies.

Genetics of Osteoarthritis and Lifestyle (GOAL) study. Large joint OA cases were recruited from hospital orthopedic surgery lists in the Nottingham area as previously described (6). Approval for recruitment was obtained from the Research Ethics Committees of Nottingham City Hos-

pital and North Nottinghamshire. Preoperative knee or pelvis radiographs of study subjects were examined to confirm the diagnosis and to grade for changes of OA (6). Radiographs were scored for individual radiographic features of OA by a single observer (SAD) and graded from 0–3 according to a standard atlas using the Kellgren/Lawrence (K/L) grade for each joint (7). Only individuals of European descent were included in the genetic study. Subjects ages 45–85 years who had undergone intravenous urography in the same hospital were recruited as controls and underwent clinical examination and joint radiographs. Study subjects also completed a WOMAC pain score (range 0–20) and a joint pain questionnaire. Individuals who had radiographic evidence of knee OA, defined as a K/L grade ≥2, were included as knee OA cases for the association analysis between the WOMAC and rs6746030. Total knee replacement (TKR) cases were classified as symptomatic OA regardless of current pain status. Individuals who had not undergone a TKR or a total hip replacement and presented current knee pain 15 or more days of a month during the past year were classified as symptomatic OA, or otherwise were classified as asymptomatic. Multiple regional pain was defined as pain over the past year in the axial skeleton in addition to pain in joint regions in all 4 body quadrants.

Medical Research Council Hertfordshire Cohort Study (HCS). The HCS is a large population-based study. Details of the study design have been published previously (8). Subjects were recruited and attended a clinic for further investigation; a subgroup (498 men and 489 women) underwent knee radiographs, of which 767 completed a knee pain questionnaire (including the WOMAC pain questionnaire) and had DNA available for the present study. Ethical approval was obtained from the East and North Hertfordshire Ethical Committees. Knee radiographs were graded using a standard atlas and the K/L score was determined (7). For the knee OA section of the study, subjects with a K/L grade ≥2 at one or both knees were included and were classified as having painful OA if they reported pain on most days in the last month. Regardless of the availability of radiographs, 824 individuals from the HCS with DNA and detailed pain questionnaire information were classified as affected or unaffected by multiple regional pain. Affected status was defined as having pain over the past year in parts of their bodies on both sides of the body (left and right) and above and below the waist in addition to axial skeletal pain, and this pain had lasted for 3 or more months.

The Clearwater Osteoarthritis Study (COS). The COS is an ongoing community-based cohort study designed to identify risk factors for the development and progression of OA. A complete description of the COS has been previously published (9). The COS was approved by the community-based Institutional Review Board of the Arthritis Research Institute of America, an uncompensated, nonemployee board that has representation from the medical disciplines. Study participants of both sexes ages 40 years and older, with or without OA, have been included. Exclusion criteria were self-reported rheumatic disease

Table 1. Descriptive characteristics of the study subjects*			
	GOAL	**HCS**	**COS**
Knee OA			
Symptomatic radiographic OA, n	244	129	71
Age, mean ± SD years	65.4 ± 8.5	66.0 ± 2.7	65.6 ± 8.7
Female sex, %	40.65	58.3	67.1
BMI, mean ± SD kg/m^2	30.3 ± 5.0	29.0 ± 5.2	28.7 ± 5.1
WOMAC pain score, mean ± SD	7.7 ± 4.5	6.3 ± 3.9	N/A
Na$_V$1.7 R1150W, %	15.35	16.3	8.6
K/L grade >2, %†	89.70	15.4	30.0
TKR, n	851	8	22
Age, mean ± SD years	69.0 ± 6.8	66.5 ± 1.97	66.7 ± 7.9
Female sex, %	46.88	37.5	63.6
BMI, mean ± SD kg/m^2	31.4 ± 5.3	28.1 ± 3.0	30.5 ± 5.4
WOMAC pain score, mean ± SD	5.1 ± 4.6	4.5 ± 6.2	N/A
Na$_V$1.7 R1150W, %	13.37	12.5	13.6
K/L grade >2, %	97.80	75	77.3
Asymptomatic radiographic OA, n	316	130	83
Age, mean ± SD years	67.7 ± 7.3	65.0 ± 2.7	63.9 ± 9.6
Female sex, %	43.00	46.9	54.7
BMI, mean ± SD kg/m^2	28.5 ± 4.9	26.6 ± 3.4	26.1 ± 5.0
WOMAC pain score, mean ± SD	0.6 ± 1.8	0.7 ± 1.4	N/A
Na$_V$1.7 R1150W, %	13.54	15.4	13.2
K/L grade >2, %	58.12	1.5	15.7
Multiple regional pain			
Affected, n	169	35	30
Age, mean ± SD years	67.2 ± 7.5	65.9 ± 2.3	59.3 ± 10.3
Female sex, %	60.9	80.0	76.7
BMI, mean ± SD kg/m^2	31.8 ± 5.8	29.0 ± 5.4	29.5 ± 6.1
WOMAC pain score, mean ± SD	8.4 ± 4.7	6.2 ± 4.7	N/A
Na$_V$1.7 R1150W, %	18.6	17.1	16.7
Unaffected, n	2,972	789	300
Age, mean ± SD years	66.5 ± 7.9	65.0 ± 2.7	60.2 ± 9.1
Female sex, %	48.0	53.3	69.1
BMI, mean ± SD kg/m^2	29.1 ± 5.2	26.8 ± 4.1	26.8 ± 4.8
WOMAC pain score, mean ± SD	3.2 ± 4.3	2.3 ± 3.3	N/A
Na$_V$1.7 R1150W, %	14.2	14.1	10.5

* GOAL = Genetics of Osteoarthritis and Lifestyle study; HCS = Hertfordshire Cohort Study; COS = Clearwater Osteoarthritis Study; OA = osteoarthritis; BMI = body mass index; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; N/A = not available; K/L = Kellgren/Lawrence; TKR = total knee replacement.
† Highest K/L grade at either the patellofemoral or tibiofemoral compartment of either knee.

(other than OA), disabling neuralgic disease, wheelchair dependence, or mental incompetence. Height and weight were assessed and subjects completed a questionnaire detailing, among other things, demographics, and a module describing joint symptoms. Weight-bearing anteroposterior view radiographs of both knees were taken at the first and all subsequent examinations and graded using an atlas (7). For this study, 176 individuals with a K/L grade ≥2 at visits 1 or 2 were included and compared for their painful knee status. Multiple regional pain was defined as current pain that the patient considered as handicapping both above and below the waist in addition to axial skeletal pain.

Laboratory methods. For the COS participants, buccal swab samples were collected by mail using Isohelix swabs (Cell Projects). Stabilizing solution was added according to the manufacturer's instructions, and the brushes were then frozen and shipped via courier for DNA extraction, which was carried out using standard protocols. For the GOAL and HCS participants, genomic DNA was extracted from peripheral blood, and DNA from the GOAL cohort was further whole-genome amplified using standard protocols. Genotyping of the rs6746030 SNP was carried out by KBioscience. The SNP was genotyped using the KASPar chemistry, a competitive allele-specific polymerase chain reaction SNP genotyping system. The polymorphism was in Hardy-Weinberg equilibrium in the controls ($P > 0.05$).

Statistical analysis. The association between the WOMAC and rs6746030 was tested using linear regression, and TKR and radiographic cases were analyzed separately. The association between rs6746030 and symptomatic OA and with multiple regional pain was assessed by a logistic regression model. Effect estimates (ln of the odds ratio [OR] or linear regression parameter [β]) and their SEs were estimated independently within each study, adjusted for age, sex, and body mass index for all analyses. Analy-

ses on the WOMAC and symptomatic knee pain were also adjusted for K/L grade. Meta-analyses of the results of the different cohorts were carried out in R, version 2.10.1 (R Foundation for Statistical Computing, online at http://www.r-project.org/). Heterogeneity was evaluated with the Q statistic and I^2. Random-effects models were not used because no heterogeneity was seen for any of the analyses ($I^2 = <10\%$).

The statistical power for each of the 3 traits was calculated given a minor allele frequency of 14% of the risk allele and the sample sizes available (Table 1), assuming a log-additive (codominant) genetic model with an alpha level of 0.05 using Quanto, version 1.2.4 (University of Southern California, online at http://hydra.usc.edu/gxe). The traits investigated were symptomatic versus asymptomatic knee OA, multiple regional pain, and the WOMAC.

Results

The descriptive characteristics of the study cohorts, including the mean $\pm$ SD WOMAC scores and the number of samples available for each study, are shown in Table 1. Statistical power for the WOMAC analysis in the combined HCS and GOAL radiographic knee OA samples given the observed minor allele frequency was 78% and 75%, respectively, for the TKR samples for an alpha level of 0.05 with an effect size of $\beta = 0.76$ as published (4), given the observed distribution of WOMAC scores among these two categories of OA cases. For the analysis comparing symptomatic versus asymptomatic OA, given the number of symptomatic and asymptomatic cases, an OR of 1.32 for the 1150W variant is needed for 80% power under a codominant model with alpha level of 0.05. For multiple regional pain, given the number of affected and unaffected individuals, an OR of 1.42 is needed to achieve 80% power with an alpha level of 0.05. Therefore, the present study is powered to replicate effect sizes similar to those in the original report on WOMAC pain scores, and for symptomatic versus asymptomatic OA and multiple regional pain, it requires moderate genetic effect sizes.

The 1150W variant was not significantly associated with WOMAC scores among radiographic knee OA patients from the HCS or GOAL ($\beta = 0.21$ [95% confidence interval (95% CI) $-0.37, 0.79$], $P = 0.46$) or among TKR cases ($\beta = 0.21$ [95% CI $-0.41, 0.82$], $P = 0.51$). A meta-analysis of radiographic samples with the original published study (4) gives a nominally statistically significant result (Figure 1A), with a P value of 0.030.

No association between the 1150W variant and symptomatic OA was seen comparing symptomatic and asymptomatic OA cases in the COS, HCS, and GOAL and adjusting for radiographic severity (K/L grade), yielding an OR of 0.9 (95% CI 0.71, 1.15; $P = 0.38$) (Figure 1B). Results remained not significant when cases were stratified by K/L grade, resulting in an OR of 1.09 (95% CI 0.75, 1.59; $P = 0.65$) for cases with a K/L grade of 2 and an OR of 0.85 (95% CI 0.63, 1.16; $P = 0.28$) among cases with a K/L grade ≥ 3. No heterogeneity was seen between cohorts in any of these analyses.

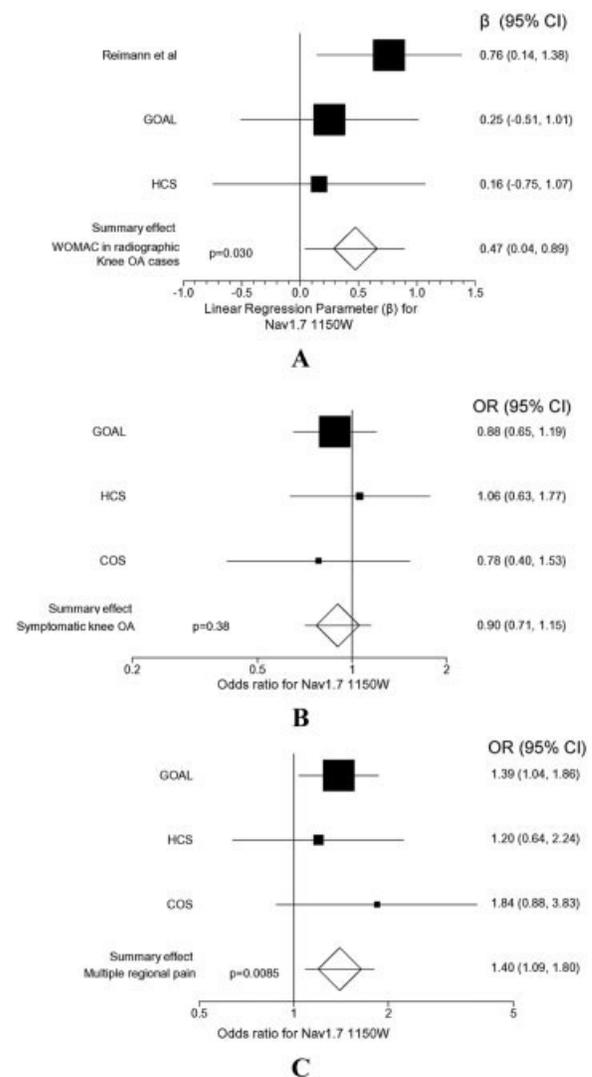On the other hand, a significant association was found

Figure 1. Forest plot of study-specific estimates and fixed-effects summary effect size and 95% confidence intervals (95% CIs) for the association between the Na$_V$1.7 R1150W variant and **A**, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores in radiographic osteoarthritis (OA) cases in the current and previously published studies, **B**, symptomatic versus asymptomatic knee OA, and **C**, multiple regional pain. All effect size estimates are adjusted for age, sex, and body mass index. Effect size estimates for the WOMAC and symptomatic OA are also adjusted for radiographic severity (Kellgren/Lawrence grade). GOAL = Genetics of Osteoarthritis and Lifestyle study; HCS = Hertfordshire Cohort Study; OR = odds ratio; COS = Clearwater Osteoarthritis Study.

between the 1150W variant and multiple regional pain in the GOAL study (OR 1.39 [95% CI 1.04, 1.86], $P < 0.026$). The overall effect was statistically significant, with an OR of 1.40 (95% CI 1.09, 1.80; $P = 0.0085$) when data from all 3 studies were analyzed (Figure 1C), and no interstudy heterogeneity was detected ($I^2 = 0\%$).

Discussion

In this study, the 1150W Na$_V$1.7 variant was not associated with WOMAC pain in knee OA patients from two UK cohorts, although there was a nonsignificant trend in the

same direction as that of the previously published study (4). We found no effect of this variant on symptomatic versus asymptomatic knee OA in all 3 study cohorts overall or when stratifying by radiographic severity. However, we did observe an association with multiple regional pain.

Failure to replicate the study by Reimann and coworkers (4) might be due to differences in ascertainment of the cohorts. Nevertheless, the association of WOMAC pain scores was not significant in either the HCS or the GOAL cohort among radiographic cases after adjusting for radiographic severity, nor among TKR cases. This is consistent with the fact that we find no evidence for a role of the 1150W variant in symptomatic OA when symptomatic OA cases are compared to people with asymptomatic OA, where we failed to find even a trend for the variant allele to predispose to symptomatic OA except among cases with a K/L grade of 2. The trend in WOMAC scores is in agreement with a role of the variant allele at *SCN9A* in determining higher pain sensitivity, which is not necessarily OA specific. Given the CIs of the genetic effect estimates obtained by us, a very small role of the risk of symptomatic OA (OR ≤1.10) cannot be excluded. Therefore, it is possible for a study with 5,000 asymptomatic cases and 10,000 symptomatic cases to detect an OR of 1.10 as statistically significant with 80% power for this variant.

Other study limitations include the different definitions of multiple regional pain across the cohorts. In two of the cohorts (COS and GOAL), the definition relies on a widespread distribution of pain compatible with chronic widespread pain (pain in the axial skeleton, above and below the waist, right and left sides of the body), whereas it was not possible to apply this stricter definition to the COS. Additional differences between the cohorts are that in the GOAL and COS, the definition is based on joint regional pain, whereas in the HCS it is on body parts with pain. Nevertheless, in all 3 studies we find a higher prevalence of the variant involved in higher $Na_V1.7$ activity among affected individuals and no interstudy heterogeneity. Therefore, our data, in the context of the previous study (4), provide support for a role of this variant in increased pain perception, but apparently this is not related in a major way to knee OA–specific pain.

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