

# Identification of Genetic Variants Associated with Painful Osteoarthritis – Results from a Community Based Genome-Wide Association Study

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## INTRODUCTION

- Osteoarthritis (OA) is a highly prevalent joint disorder and one of the most common causes of pain, but individual susceptibility to painful symptoms associated with OA is not well understood.
- We searched for genetic polymorphisms that associate with pain report in individuals with symptomatic (painful) knee and/or hip OA compared to individuals with asymptomatic knee and/or hip OA.
- After comparing baseline phenotypes in the overall study population to phenotypic characteristics specifically associated with pain, we used a genome wide analysis to evaluate genetic characteristics associated with pain phenotypes.

## SUBJECTS

- Subjects**
- Following QA/QC, final GWAS dataset included 2,081 subjects of both sexes and two racial/ethnic groups (European ancestry, African-American), and with only one individual from each family cluster. Subset of the larger cohort (N = 2,754).
- Age ≥ 45 yrs; mean age 63.1 (sd 10.6)

*Selected cohort characteristics according to the presence of symptomatic OA in at least one joint (K-L score of 2 or more and at least mild pain on most days).*

Characteristic	Yes	No	Yes	No
Age	1123	421	182	1119
Female	1091	391	163	1000
Race	1951	297	93	1000
Body Mass Index	1000	373	1.9	1.9
Arthritis and Tendinitis (ATD)	Yes	1726	7.6	13.2
Arthritis and Tendinitis (ATD)	No	198	7.0	13.2
Chronic Symptom (CSIS)	Yes	1726	17.1	13.2
Chronic Symptom (CSIS)	No	198	17.4	13.2
Chronic Pain	Yes	1726	17.1	13.2
Chronic Pain	No	198	17.4	13.2
WOMAC pain	Yes	1127	3.2	4.4
WOMAC pain	No	198	7.0	4.3

### Pain and OA by knee and hip joint.

OA	Left Hip		Right Hip	
	Pain	No Pain	Pain	No Pain
OA	217	454	651	565
No OA	523	1272	1793	1883
	740	1726	2444	2448
% with Pain	29%	% with Pain	23%	23%
% with OA	27%	% with OA	23%	23%
% with Pain among OA	29%	% with Pain among OA	26%	26%
Odds of Pain if OA	1.221	Odds of Pain if OA	1.232	1.232
RR of Pain if OA	1.147	RR of Pain if OA	1.168	1.168

OA	Left Knee		Right Knee	
	Pain	No Pain	Pain	No Pain
OA	447	256	703	690
No OA	718	1258	1366	1951
	1165	1504	2069	2631
% with Pain	46%	% with Pain	44%	46%
% with OA	26%	% with OA	26%	26%
% with Pain among OA	38%	% with Pain among OA	38%	38%
Odds of Pain if OA	3.885	Odds of Pain if OA	3.885	3.885
RR of Pain if OA	3.741	RR of Pain if OA	3.741	3.741

## Clinical Assessment

- Clinical and functional exam, including radiographic assessment of knee and hip
- Two administered home interviews
- An extensive battery of questionnaires to characterize arthritis, including Western Ontario and MacMaster Osteoarthritis Index (WOMAC); self-report of pain; and psychosocial profile including anxiety, depression, optimism, and social support.

## Biological Sampling

- Sample of whole blood for genotyping.

## GENOTYPING

### Genotyping performed using the Illumina 1M-Duo platform

- Assays over one million single nucleotide polymorphisms (SNPs) across the entire genome targeted at genes linked to biological pathways known to influence pain neurotransmission, inflammation, and mood/affect
- SNP choice was weighted toward putatively functional SNPs (i.e., non-synonymous changes, coding regions, promoter regions, splice junctions)
- High linkage disequilibrium (LD) coverage of complete gene loci
- For more information from Illumina, Inc. (San Diego, CA): <http://www.illumina.com>

### Genotyping and sample quality assessment /quality control

- Samples were rejected due to:

- call rate <98% (overall call rate for this dataset = 0.99551)
- relatedness between samples evidenced by identity-by-state (IBS) analysis
- unreconcilable disagreement between self-reported and genotypic sex and/or race
- SNP markers were eliminated based on:
  - call rate <98%
  - low minor allele frequency (<0.005)
  - repeatability across duplicated control samples (<99.9%)
  - Hardy-Weinberg disequilibrium and visual examination of genotyping cluster plots were used to detect possible calling errors.

These datasets included 1,065,734 SNPs (92.3% of probes attempted).

#### SNP Filter Results:

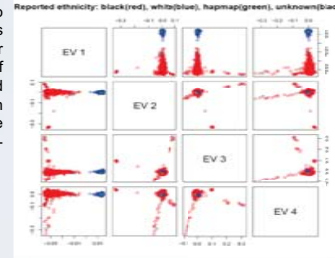
Filter	SNPs	SNPs
SNPs attempted	1,065,734	1,065,734
Initially only probes	44,006	11,581,871
MAF < 0.005	49,000	1,105,188
Call rate < 98%	1,171	10,507,771
MAF < 0.005	2,208	10,505,563
MAF < 0.005	23,611	10,503,192
Discordant rate > 0	23,611	10,503,192
Total	13,843	10,503,192
Percent	7.70	92.30

#### Sample Filter Results:

Filter	SNPs	SNPs
Genotype	50	2,953
Gender/Race/Heterozygosity	50	2,953
mismatches	17	3,026
Misreadiness	445	2,081
Relatedness	912	2,081
Total	1,576	80,215

## GENOTYPING/SAMPLE QUALITY CONTROL

We applied principal components analysis to IBS information to cluster study individuals by ancestry group. These MDS plots in four dimensions allow for visual inspection of genetic clusters of individuals with shared racial composition. Subjects have been colored according to reported race (blue=European ancestry, red=African-American).



## PAIN PHENOTYPES AND ASSOCIATIONS

We looked for associations using three definitions of OA explored in a multiracial population with the appropriate correction for population stratification:

- presence of pain determined by WOMAC (using WOMAC score > 0 and WOMAC score ≥ 3 as thresholds for positive indication of pain)
- self-reported pain rating using WOMAC score as a continuous variable
- endorsement of "mild or greater" or "moderate or greater" joint pain in the knee or hip on most days.

### WOMAC of 0 vs. WOMAC > 0

(N=1190 cases, 737 controls)

Chr 3: several significant SNPs (rs16839940) between

GPR15 (G protein-coupled receptor 15)

CPOX (coproporphyrinogen oxidase)

Chr 11: several significant SNPs (rs1893011, rs1939852, rs2466584, rs2512295) in numerous olfactory receptor genes:

OR10G7, OR8G2, OR8G1, OR8D1

Chr 5: no known genes

Chr 12: FAM19A2

When adjusted for BMI and KL score, candidate pain genes TACR1 and PRKG1 emerge.

### WOMAC of 0 vs. WOMAC ≥ 3

(N=982 cases, 737 controls)

Significant results similar to above hits:

Chr 3: several significant SNPs between

GPR15 (G protein-coupled receptor 15)

CPOX (coproporphyrinogen oxidase)

Chr 11: numerous olfactory receptor genes

Chr 5: no known genes

A new significant SNP emerges: Chr 4: ANKRD17

### Regression on the number of painful joints

(2,077 joints)

Two significant results:

Chr 16: A2BP1 (ataxin 2-binding protein 1); rs17139085; rs1716508

Chr 19: ZNF234 (zinc finger protein 234)

Other suggestive SNPs:

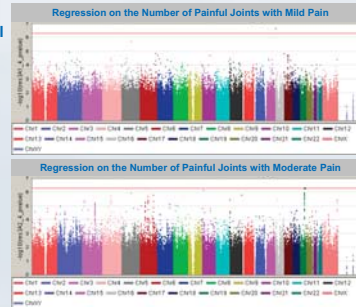
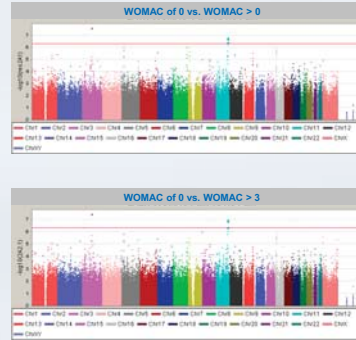
Chr 2: LRRFIP1 (leucine rich repeat (in FLII) interacting)

Chr 17: SMTNL2 (smoothelin-like 2)

Chr 7: NXP1 (neurexophilin-1)

Chr 17: ABCA8 (ATP-binding cassette sub-family A member 8)

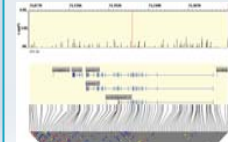
Chr 10: PAOX (polyamine oxidase [exo-N4-amino])



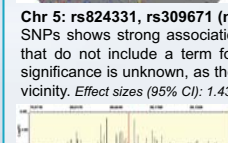
## SINGLE SNP ASSOCIATIONS

The following SNPs were found to be significantly associated with at least one tested phenotype using a validated threshold of  $p=5 \times 10^{-7}$ .

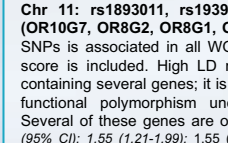
**Chr 3: rs16839940 (GPR15-CPOX):** The effect of this SNP was observed most strongly in the dichotomous (pain vs. no pain) WOMAC test, in the full population and without corrections for BMI or KL. *Effect size (95% CI): 1.47 (1.21-1.78)*



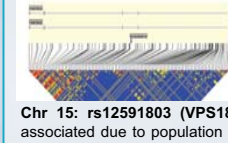
**Chr 4: rs17179238 (ANKRD17):** This SNP is most highly associated in the tests of WOMAC as a dichotomous variable with a cutoff of 3. *Effect size (95% CI): N/A*



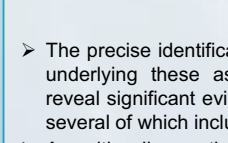
**Chr 5: rs824331, rs309671 (no known genes):** This pair of SNPs shows strong association in all of the WOMAC tests that do not include a term for KL score. Their functional significance is unknown, as there are no known genes in the vicinity. *Effect sizes (95% CI): 1.43 (1.16-1.77); 1.43 (1.15-1.78)*



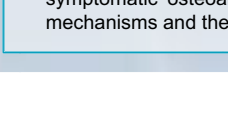
**Chr 10: rs1655622, rs1655623 (no known genes):** This pair of SNPs is also located in a gene desert. Their effect is observed in all WOMAC tests, however, and most strongly observed in the Europeans-only group with BMI included as a covariate. *Effect sizes (95% CI): 1.36 (1.11-1.67); 1.35 (1.10-1.66)*



**Chr 11: rs1893011, rs1939852, rs2466584, rs2512295 (OR10G7, OR8G2, OR8G1, OR8D1):** This cluster of linked SNPs is associated in all WOMAC tests, except when KL score is included. High LD region extends over an area containing several genes; it is difficult to distinguish the true functional polymorphism underlying these associations. Several of these genes are olfactory receptors. *Effect sizes (95% CI): 1.55 (1.21-1.99); 1.55 (1.21-1.98); 1.56 (1.21-2.02); 1.55 (1.21-1.99)*



**Chr 12: rs1087755 (FAM19A2):** This SNP is common in African-American subjects but has very low frequency (MAF<0.01) in European ancestry samples. In tests on African-American samples only, this SNP was strongly associated ( $p<5 \times 10^{-7}$ ) with WOMAC; this may exert a race-specific effect on pain ratings. *Effect size (95% CI): N/A*



## CONCLUSIONS

- The precise identification of the genes and functional polymorphisms underlying these associations is ongoing. However, our findings reveal significant evidence for association in seven genomic regions, several of which include testable candidate genes.
- As with all genetic association studies, our results need to be replicated in independent samples.
- These findings constitute the first genome-wide analysis of symptomatic osteoarthritis and may lead to the discovery of novel mechanisms and therapeutic treatments for painful OA.

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