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)

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.

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).

	Symptomatic OA	N	Mean	SD	t	sig
Age	No	1753	62.1	10.2	-11.0	.000
	Yes	1001	66.8	10.5		
Body Mass Index	No	1751	29.7	5.9	-9.3	.000
	Yes	1000	32.3	7.6		
Anxiety and Tension (AMSD)	No	1736	7.2	1.9	3.2	.001
	Yes	998	7.0	2.0		
Depressive Symptoms (CES-D)	No	1734	6.3	8.6	-3.2	.001
	Yes	983	7.4	8.4		
Social Support	No	1746	17.8	3.0	.2	.862
	Yes	996	17.8	3.1		
Optimism (LOT)	No	1741	30.8	4.3	2.4	.014
	Yes	980	30.3	4.1		
WOMAC_pain	No	1737	3.2	4.4	-20.1	.000
	Yes	990	7.0	4.9		

Pain and OA by knee and hip joint.

Left Hip

	Pain	No Pain	
OA	217	434	651
No OA	521	1272	1793
	738	1706	2444
% with Pain			30%
% with OA			27%
% with Pain among OA			29%
Odds of Pain if OA			1.221
RR of Pain if OA			1.147

Right Hip

	Pain	No Pain	
OA	198	367	565
No OA	567	1316	1883
	765	1683	2448
% with Pain			31%
% with OA			23%
% with Pain among OA			26%
Odds of Pain if OA			1.252195
RR of Pain if OA			1.163815

Left Knee

	Pain	No Pain	
OA	447	256	703
No OA	718	1248	1966
	1165	1504	2669
% with Pain			44%
% with OA			26%
% with Pain among OA			38%
Odds of Pain if OA			3.035
RR of Pain if OA			1.741

Right Knee

	Pain	No Pain	
OA	441	239	680
No OA	722	1229	1951
	1163	1468	2631
% with Pain			44%
% with OA			26%
% with Pain among OA			38%
Odds of Pain if OA			3.140909
RR of Pain if OA			1.752467

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 = 0.999551)
  - 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:

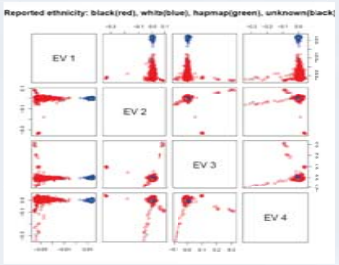
Filter	SNPs dropped	SNPs remaining
SNPs attempted		1199187
Intensity only probes	44496	1154691
MAF = 0	49503	1105188
Call rate < 98%	11711	1093477
HWE p < 10^-6	2238	1091239
MAF < 0.005	23161	1068078
Discordant rate > 0	2344	1065734
Total	133453	1065734
percent	7.70	92.30

Sample Filter Results:

Filter	Samples dropped	Samples remaining
Genotyped		2593
Gender/Race/Heterozygosity mismatches	50	2543
Missingness	17	2526
Relatedness	445	2081
Total	512	2081
percent	19.75	80.25

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

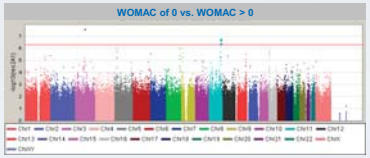
We looked for associations using three definitions of OA explored in a multiracial population with the appropriate correction for population stratification:

1) presence of pain determined by WOMAC (using WOMAC score > 0 and WOMAC score ≥ 3 as thresholds for positive indication of pain)
2) self-reported pain rating using WOMAC score as a continuous variable
3) 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)

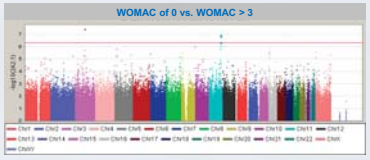
Chr 3: several significant SNPs (rs16839940) between
GPR15 (G protein-coupled receptor 15)
CPOX (coproporphyrinogen oxidase)
Chr 11: several significant SNPs (rs1893011, rs1939852, rs2466584, rs2512295) in numerous olfactory receptor genes:
OR10G7, OR8G2, OR8G1, OR8D1
Chr 5: no known genes
Chr 12: FAM19A2
When adjusted for BMI and KL score candidate pain genesTACR1 and PRKG1 emerge.



WOMAC of 0 vs. WOMAC ≥ 3

(N=982 cases, 737 controls)

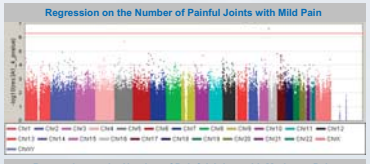
Significant results similar to above hits:
Chr 3: several significant SNPs between
GPR15 (G protein-coupled receptor 15)
CPOX (coproporphyrinogen oxidase)
Chr 11: numerous olfactory receptor genes
Chr 5: no known genes
A new significant SNP emerges: Chr 4: ANKRD17

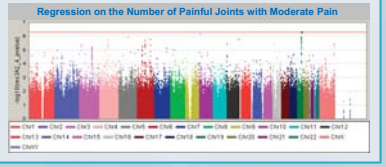


Regression on the number of painful joints

(2,077 joints)

Two significant results:
Chr 16: A2BP1 (ataxin 2-binding protein 1); rs17139085; rs716508
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: NXPH1 (neurexophilin-1)
Chr 17: ABCA8 (ATP-binding cassette sub-family A member 8)
Chr 10: PAOX (polyamine oxidase [exo-N4-amino])





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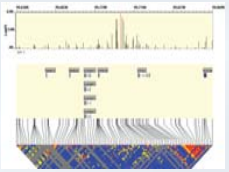
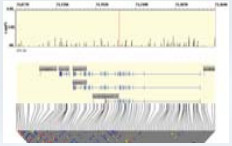
SINGLE SNP ASSOCIATIONS

The following SNPs were found to be significantly associated with at least one tested phenotype using a validated threshold of $p=5x10^{-7}$.

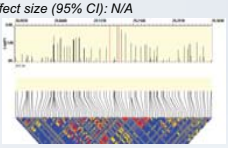
Chr 3: rs16839940 (GPR15-CPOX): The effect of this SNP was observed most strongly in the dichotomous (pain vs. no pain) WOMAC test, in the full population and without corrections for BMI or KL. *Effect size (95% CI): 1.47 (1.21-1.78)*

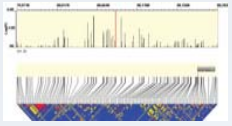




Chr 4: rs17179238 (ANKRD17): This SNP is most highly associated in the tests of WOMAC as a dichotomous variable with a cutoff of 3. *Effect size (95% CI): N/A*

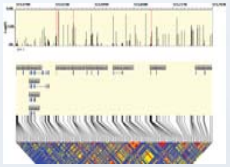
Chr 5: rs824331, rs309671 (no known genes): This pair of SNPs shows strong association in all of the WOMAC tests that do not include a term for KL score. Their functional significance is unknown, as there are no known genes in the vicinity. *Effect sizes (95% CI): 1.43 (1.16-1.77) ; 1.43 (1.15-1.78)*

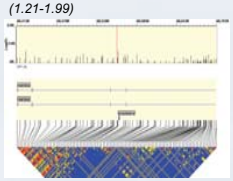




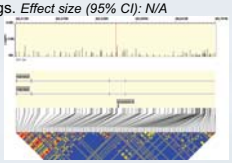
Chr 10: rs1655622, rs1655623 (no known genes): This pair of SNPs is also located in a gene desert. Their effect is observed in all WOMAC tests, however, and most strongly observed in the Europeans-only group with BMI included as a covariate. *Effect sizes (95% CI): 1.36 (1.11-1.67); 1.35 (1.10-1.66)*

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: rs10877755 (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 ($p<5x10^{-7}$) with WOMAC; this may exert a race-specific effect on pain ratings. *Effect size (95% CI): N/A*



Chr 15: rs12591803 (VPS18): This SNP appears to be associated due to population stratification. It is prevalent in African-Americans (MAF>0.4) but very rare in European ancestry samples (MAF<0.01); no European ancestry cases have the minor allele. *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.