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GENE-BASED ANALYSIS OF A METAANALYSIS OF OSTEOARTHRITIS GENOME WIDE ASSOCIATION STUDIES
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Purpose: There is ample evidence of the insufficiency of current approaches to discover a large fraction of the genetic component of complex diseases. Part of this missing component is already present in the GWAS as multiple SNPs showing small differences between cases and controls. The contribution of these multiple SNPs has been shown in studies of OA among other diseases. However, we do not have validated tools to distinguish between genuine association and random noise at these weak association levels. It is possible that using genes or pathways as the analysis units could uncover part of this polygenic component. However, reported Gene Based Analyses (GBA) have limitations in important aspects like definition of gene boundaries, consideration of linkage disequilibrium between SNPs and validation of significance thresholds. In addition, none of the previous studies has been replicated in independent samples. Confronted with this situation, we have tried to address the limitations of GBA to apply it to research in OA genetics.

Methods: We have developed a procedure for GBA that incorporates definition of genes according with the standardized recombination rate (SRR) from the deCODE genetic map, a process for decorrelation of the P values pertaining to each SNP in the gene that is based in iterative and ordered Cholesky decomposition of the \( R^2 \) matrix, an evaluation of the goodness of fit between the original P values and the predicted from the decorrelated P values, estimation of a unique summary statistic for each gene, and replication in additional samples. This GBA has been applied to the meta-analysis of 9 OA GWAS (partly reported). Six separate analyses were done for data stratified by the 2 x 3 table of joint x gender with classes: knee and hip for joint and women, men and combined for gender. All analyses were done in a cluster of Unix nodes (SVG at the CESGA super-computing centre).

Results: Application of the SRR-based definition of genes led to inclusion of 5’ and 3’ sequences extensions of variable lengths with median of 45.3 Kb (IQR = 22.6-90.2 with sex averaged SRR). We also defined a set of “co-genes” as the fusion of genes overlapping by more than 50%. Decorrelation was obtained for 94.6 - 95.2% of the 18022 genes with good accuracy as defined by analysis based in Mahalanobis distances. Results showed multiple associations four of them below the genome-wide significance threshold (5 x 10^{-8}). Others were below alternative thresholds that could be appropriate given the lower number of independent tests in a GBA than in a GWAS. Therefore we considered the 32 results shown in Table 1 worth pursuing for replication.

Table 1

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Threshold</th>
<th>Knee OA</th>
<th>Hip OA</th>
<th>Women</th>
<th>Men</th>
<th>All</th>
<th>Women</th>
<th>Men</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>GWAS</td>
<td>(&lt; 5 x 10^{-8})</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>genes x strata</em></td>
<td>(&lt; 5 x 10^{-5}) to (&lt; 5 x 10^{-3})</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>genes</em></td>
<td>(&lt; 3 x 10^{-5}) to (&lt; 5 x 10^{-3})</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>co-genes</em></td>
<td>(&lt; 7 x 10^{-5}) to (&lt; 3 x 10^{-3})</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

They included three associations highlighted in previous studies: GDF5 with knee OA in women (top association \( P = 1.9 \times 10^{-8}\) obtained considering a co-gene), COL11A1 with hip OA in the combined analysis (top association also with a region including overlapping genes, \( P = 2.2 \times 10^{-10}\) and SPP1/HNRNQC with hip OA in men (\( P = 3.5 \times 10^{-10}\) ). These results do not amount to independent replication because of overlap with previous studies. All the other associations selected for replication are novel.

Conclusions: A new GBA approach has been developed that through successful decorrelation of most SNPs in a GWAS OA metaanalysis has allowed finding new associations. These results are very promising because they include top associations below the GWAS threshold for significance and because it has retrieved three loci that were highlighted in previous OA studies. Therefore, this GBA strategy seems to perform well as an extension of the GWAS. Validation of 32 associations by replication with data from an independent large GWAS (arcGENE phase II) is under way.

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META-ANALYSIS IDENTIFIES NOVEL GENES INFLUENCING LEVELS OF THE POTENTIAL OA BIOMARKERS SCOMP AND UCTX2


Purpose: Reliable biochemical markers to sensitively monitor early OA onset and/or progression would greatly favor early prognosis and the search for effective therapies. Previous studies showed that urinary C-telopeptide of type II collagen (uCTX2) is a sensitive marker of whole body joint damage as reflected by its correlation to radiographic signs of OA at different joint sites, however, it lacks specificity. Serum cartilage oligomeric protein (sCOMP) associates with both OA and age and has a high heritable component. Insight into the innate variation of these markers within subjects may increase its possible application as OA biomarker. Here, we set out to identify novel genes influencing levels of the potential OA biomarkers SCOMP and uCTX2.

Methods: Marker levels were measured in respectively 6 studies for uCTX2 (N=3316; studies from the Netherlands: GARP, Leiden Longevity (LLS), Rotterdam Study II (RS-II) and the United Kingdom: TwinsUK, CHINGFORD, VIDEO) and 7 studies for SCOMP (N=4554; including the same studies as for uCTX2 in addition to RS-I). For the discovery, we applied a meta-analysis of genome wide association (GWA) studies for these quantitative traits based on the summary statistics and P-values using the Meta-Analysis Tool (METAL). Summary odds ratio’s (ORs) were estimated for compelling genes using the random-effects model of DerSimonian and Laird, as implemented in the R package Meta (http://www.r-project.org/). Heterogeneity was examined using the I2. Replication of SNPs with suggestive evidence for association (\( P < 10^{-5}\)) was performed by de novo genotyping in the Cohort Hip and Cohort Knee (CHECK; N=964).

Results: In the uCTX2 meta-GWA, we detected a total of 3 loci with genome wide suggestive evidence for association. The most significant association was observed for a SNP located at chromosome 8 at 43.9 MB within the CUB and sushi domain-containing protein 1 (CSMD1; OR = 1.14, \( P = 1.2 \times 10^{-5}\)). In the sCOMP meta-GWA analysis, we found in total 6 loci with genome wide suggestive evidence for association. Among them was a SNP within the mannose receptor C type 1 (MRC1) gene with genome wide significance (OR = 0.78, \( P = 2.5 \times 10^{-10}\)). Of note was the association for a SNP within the sCOMP gene itself (OR = 1.13, \( P = 2.0 \times 10^{-5}\)). Replication of these loci within CHECK resulted in additional confirmation with a similar effect and with significant statistical evidence for the uCTX2 gene CSMD1 (\( P = 0.032\)) and a suggestive evidence for the sCOMP gene MRC1 (\( P = 0.066\)).

Conclusions: By applying GWAS meta-analysis, we are the first to identify a locus influencing innate levels of sCOMP with genome wide significance. This, and other loci associated with uCTX2 and sCOMP levels will be studied and compared to uCTX2 and sCOMP itself for their potential as biomarkers to detect OA at an early stage. In addition, follow-up research will be aimed at studying their sensitivity and specificity in association with OA phenotypes and the expression of these genes in relevant tissues.