Meta-analyses of genes modulating intracellular T3 bio-availability reveal a possible role for the DIO3 gene in osteoarthritis susceptibility

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ABSTRACT

Objective To study whether common genetic variants of the genes involved in the complex regulatory mechanism determining the intracellular bio-availability of T3 influence osteoarthritis onset.

Methods In total 17 genetic variants within the genes encoding WDF40-repeat/SOCS-box protein 1, ubiquitin specific protease 33, thyroid hormone receptor α, deiodinase, iodothyronine, type III (DIO3) and Indian hedgehog were measured and associated with osteoarthritis in a meta-analyses in European populations from the UK, The Netherlands, Greece and Spain containing a total of 3252 osteoarthritis cases and 2132 controls.

Results The minor allele of the DIO3 variant rs945006 showed suggestive evidence for protective association in the overall meta-analyses, which was supported by individual osteoarthritis studies and osteoarthritis subtypes. The association appeared most significant in cases with knee and/or hip with an allelic OR of 0.81 (95% CI 0.70 to 0.930) with a nominal p value of 0.004 and a permutation-based corrected p value for multiple testing of 0.039.

Conclusion The findings suggest that the DIO3 gene modulates osteoarthritis disease risk; however, additional studies are necessary to replicate our findings. To elucidate the molecular mechanisms focus should be on the local adaptation to T3 availability either during the endochondral ossification process or during ageing of the articular cartilage.

Previously, in the Genetics Osteoarthritis and Progression (GARP) study we identified the deiodinase iodothyronine type II (D2) gene (DIO2) as an osteoarthritis susceptibility gene, with this finding confirmed in other studies especially for hip osteoarthritis in women. D2 is critical in maintaining the availability of intracellular active thyroid hormone 3’3’5-triiodothyronine (T3). In the growth plate, T3 initiates the terminal differentiation of hypertrophic chondrocytes, which is important for the subsequent formation of long bones. There are, however, a number of additional interacting cellular mechanisms that modify the D2 actions. As outlined in figure 1, these include iodothyronine type III (D3) activity that depletes sources of active thyroid molecules, inactivation of D2 by ubiquination, reactivation by de-ubiquination or thyroid receptor occupancy. In the current study, we investigated 17 common genetic variants across the genes encoding WD40-repeat/SOCS-box protein 1 (WSB1), ubiquitin-specific protease 33 (USP33), thyroid hormone receptor α (THRA), deiodinase iodothyronine type III (DIO3) and Indian hedgehog (IHH) in European populations from the UK, The Netherlands, Greece and Spain in a meta-analysis containing a total of 3252 osteoarthritis cases and 2132 controls.

MATERIALS AND METHODS

Subjects Four European osteoarthritis sample collections were used from Oxford (UK), Santiago (Spain), Larissa (Greece) and Leiden (The GARP study; The Netherlands). Details of patient and control recruitment and ascertainment criteria have been described previously. Baseline characteristics and numbers of the osteoarthritis samples are shown in supplementary table 1, available online only.

SNP selection and genotyping Single nucleotide polymorphisms (SNP) were selected from the USP33, IHH, DIO3, WSB1 and THRA genes based on the successive use of HapMap tagging and SNP selector (http://snpsselector.duhs.duke.edu/hqsnps36.html). Achieved SNP coverage is shown in supplementary tables 2 and 3, available online only. Genotyping was performed by mass spectrometry using the homogeneous MassARRAY system of Sequenom (San Diego, California, USA) using standard conditions. SNP that passed the following quality control criteria were included in subsequent analyses: minor allele frequency (MAF) >0.01, Hardy–Weinberg equilibrium p>0.05 in controls. For the UK and GARP samples the genotyping rate was greater than 0.98, for the Spanish samples the genotyping rate was greater than 0.92, whereas for the Greek samples the genotyping rate was slightly lower at over 0.83. Approximately 8% of the subjects were genotyped twice and cross-checked. Error rates were low and were similar across studies.

Association strategy and power To enhance the power of our study we initially examined 5384 individuals (3252 cases with osteoarthritis at any of the joint sites available and 2132 controls) across four large European populations in a meta-analysis. At an α level of 0.0029 (0.05/number of SNP measured), population risk
0.092, a log-additive or allelic model and a MAF of 0.23 (average of the SNP allele frequencies) these studies provide an average power of 83% to detect OR higher than 1.2 (see also supplementary table 2, available online only) for the power of each individual SNP. Being aware of the clinical and genetic heterogeneity of the osteoarthritis phenotype and to avoid false-negative results, we analysed initial findings with a suggestive evidence for association further across different osteoarthritis strata.

**RESULTS**

Table 1 provides the results of the initial meta-analyses between SNP and osteoarthritis susceptibility at any joint location (knee, hip, hand) in 5384 individuals (3252 osteoarthritis cases, 2132 controls) across four European populations. Only at **DIO3** SNP rs945006 was a significant association observed for the minor allele (allele frequency 0.11) with an OR of 0.82 (95% CI 0.72 to 0.95) and a nominal p value of 0.092, a log-additive or allelic model and a MAF of 0.23 (average of the SNP allele frequencies) these studies provide an average power of 83% to detect OR higher than 1.2 (see also supplementary table 2, available online only) for the power of each individual SNP. Being aware of the clinical and genetic heterogeneity of the osteoarthritis phenotype and to avoid false-negative results, we analysed initial findings with a suggestive evidence for association further across different osteoarthritis strata.

![Diagram](image-url)

**Figure 1** Interacting cellular mechanisms that modify the deiodinase (D2) actions and that together determine intracellular T3 bioavailability. Genes encoding WD40-repeat/SOCS-box protein 1, ubiquitin-specific protease 33 (USP33), thyroid hormone receptor α (THRA), deiodinase, iodothyronine type III (DIO3) and Indian hedgehog (IHH) are depicted and tested for their association with osteoarthritis. TRE, transcription regulatory element.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Study specific and meta-analyses by allele frequency data of SNP within the genes that modify D2 actions for cases with knee, hip or hand osteoarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIO3</td>
<td>rs1109715</td>
<td>DIO3 rs1109715 OR = 0.86 (95% CI 0.72 to 1.04) p = 0.124 DIO3 rs1109715 OR = 0.93 (95% CI 0.83 to 1.05) p = 0.242 DIO3 rs1109715 OR = 1.03 (95% CI 0.84 to 1.28) p = 0.768</td>
</tr>
</tbody>
</table>
Concise report

providing suggestive evidence for the association of DIO3 SNP rs945006. We did not observe any evidence for heterogeneity of this association (p=0.544, I²=0%).

When we analysed the DIO3 SNP rs945006 for osteoarthritis at specific joint sites we observed consistent effect sizes (OR) of approximately 0.8 among the strata, which was nominally significant in the meta-analyses of cases with hip or knee osteoarthritis (p=0.006 and 0.018, respectively) and showed a similar effect size in cases with hand osteoarthritis, which was, however, not significant (p=0.201; table 2). Combining cases with either knee or hip osteoarthritis generated an OR of 0.81 (95% CI 0.70 to 0.93), with a significant nominal p value of 0.004 without evidence for heterogeneity (p=0.284, I²=21%; table 2). The permutation-based corrected p value for multiple testing of the 17 SNP was significant for this analysis with a p value of 0.039. We did not observe any sex-specific effects in the analysis that was stratified by gender (data not shown). Among cases with knee and/or hip osteoarthritis we observed an OR of 0.81 (95% CI 0.70 to 0.93), with a significant nominal p value of 0.0035 for trend and an OR of 0.80 (95% CI 0.68 to 0.95), nominal p=0.0039 when applying a dominant model. Again, no evidence for heterogeneity (p=0.511, I²=16.2% and p=0.432, I²=0%, respectively) was detected.

As the MAF of rs945006 is relatively low, at 0.11, we were not able to test for a robust recessive effect. Finally, we performed a haplotype association analyses with the DIO3 SNP measured; however, we could not detect a haplotype that delineated the effect of rs945006 further (results not shown).

DISCUSSION

By testing for association by meta-analyses across four independent European osteoarthritis cohorts, suggestive evidence was observed for a protective association of the minor allele of the DIO3 SNP rs945006. Subsequently, this association was supported in the individual osteoarthritis studies and osteoarthritis subtypes, and appeared most significant in cases with knee and/or hip osteoarthritis, with an allelic OR of 0.81 (95% CI 0.70 to 0.93) a nominal p value of 0.004 and a permutation-based corrected p value for multiple testing of 0.039.

The DIO3 gene encodes D3, which depletes active sources of thyroid and which, together with its counterpart D2, provides an elegant homeostatic mechanism to ensure intracellular T3 availability. Our current findings complement our previous outlined hypothesis that local T3 availability may contribute to osteoarthritis in two, possibly interactive, ways. In the growth plate, variation in local T3 bioavailability may cause subtle malformations of joint shape, thereby increasing the biomechanical burden on the articular cartilage with age. In articular cartilage, recuperating D2 activity with age or osteoarthritis damage may increase the propensity of these chondrocytes to become hypertrophic, exhibit cartilage suppressive expression and eventually lead to osteoarthritis onset and/or progression towards clinical outcomes.

Given the allele frequency of DIO3 rs945006, the initial power of this association was relatively low and given the number of tests performed the significance level should be considered modest, thus the observed association requires replication in additional osteoarthritis cohorts. The consistency of the effect sizes across studies and osteoarthritis subtypes, however, is adding to the credibility of the locus. Additional haplotype analyses of DIO3 SNP did not result in a more specific haplotype that delineated the observed effect further, indicating that either the SNP rs945006 itself or a SNP in perfect linkage disequilibrium may be functional. We were not able to adjust for possible confounding factors such as age and body mass index in our overall meta-analyses due to lack of data in some of the studies (supplementary table 1, available online only). For the GARP cases and controls it was shown that additional adjustment for age and body mass index did not affect the DIO3 rs945006 association, that is the SNP effect appeared more significant (p=0.04).

The DIO3 gene is located in the human imprinted region in chromosome band 14q32.2, and is preferentially but not exclusively expressed from the paternal allele during development. Moreover, the methylation status of the DIO3 gene has previously been associated with complex diseases, thus the epigenetic effects of DIO3 needs to be investigated in the context of osteoarthritis. It should also be noted that the methylation

Table 2 Study specific and meta-analyses by allele frequency data of the DIO3 SNP rs945006 stratified by joint site with osteoarthritis

<table>
<thead>
<tr>
<th>Study</th>
<th>Joint</th>
<th>DIO3 rs945006</th>
<th>Meta-analyses</th>
<th>Heterogeneity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>TG</td>
<td>GG</td>
</tr>
<tr>
<td>GARP Control</td>
<td></td>
<td>584</td>
<td>143</td>
<td>15</td>
</tr>
<tr>
<td>UK Control</td>
<td></td>
<td>654</td>
<td>182</td>
<td>10</td>
</tr>
<tr>
<td>Spanish Control</td>
<td></td>
<td>175</td>
<td>52</td>
<td>4</td>
</tr>
<tr>
<td>Greek Control</td>
<td></td>
<td>232</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td>GARP Hip</td>
<td></td>
<td>93</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>UK Hip</td>
<td></td>
<td>969</td>
<td>232</td>
<td>13</td>
</tr>
<tr>
<td>Spanish Hip</td>
<td></td>
<td>220</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>Greek Hip</td>
<td></td>
<td>46</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>GARP Knee</td>
<td></td>
<td>134</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>UK Knee</td>
<td></td>
<td>354</td>
<td>78</td>
<td>3</td>
</tr>
<tr>
<td>Spanish Knee</td>
<td></td>
<td>207</td>
<td>46</td>
<td>2</td>
</tr>
<tr>
<td>Greek Knee</td>
<td></td>
<td>180</td>
<td>41</td>
<td>4</td>
</tr>
<tr>
<td>GARP Knee/hip</td>
<td></td>
<td>191</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>UK Knee/hip</td>
<td></td>
<td>1323</td>
<td>310</td>
<td>18</td>
</tr>
<tr>
<td>Spanish Knee/hip</td>
<td></td>
<td>427</td>
<td>83</td>
<td>3</td>
</tr>
<tr>
<td>Greek Knee/hip</td>
<td></td>
<td>226</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td>GARP Hand</td>
<td></td>
<td>182</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Spanish Hand</td>
<td></td>
<td>170</td>
<td>46</td>
<td>3</td>
</tr>
</tbody>
</table>

*OR are presented with the minor allele (as indicated in supplementary table 2, available online only) as the risk allele.
†p Value adjusted for family relationships among the sibling-pairs of the Dutch Genetics Osteoarthritis and Progression (GARP) study (see also footnote to table 1). 95% CI.
‡Heterogeneity across the osteoarthritis studies quantified by the I² statistic for inconsistency whereas its statistical significance was determined with the χ² distributed Cochran Q statistic. 15
DIO3, deiodinase iodothyronine type III; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
status could have affected the currently detected association, especially in heterozygous individuals. To characterize the accounted effect of the genetic variation at the DIO3 gene in combination with these epigenetic phenomena expression analyses should be performed in normal and in osteoarthritis articular cartilage.

None of the other genes investigated showed consistent effect across studies or osteoarthritis subtypes or nominal significant associations in our meta-analyses, indicating that those genes may not contribute to the genetic susceptibility of osteoarthritis. Alternatively, there may be a lack of power or insufficient SNP coverage of the genes investigated to detect such susceptibility. Furthermore, in our gene selection we have not focused on thyroid transporter genes, which could also play an additional rate-limiting effect on intracellular T3 bioavailability.

In summary, we screened the intracellular thyroid signaling pathway for association with various osteoarthritis subtypes. The observed association with a polymorphism at DIO3 reported here, in combination with the previous observed association with a polymorphism at DIO2, adds to the credibility that local thyroid hormone availability plays a role in the aetiology of symptomatic osteoarthritis. Further research into modulators of the thyroid pathway and the influence of age-related changes are necessary to explore possible strategies for intervention. As it has been found that SNP within DIO2 and DIO3 do not affect circulating thyroid hormone levels in humans, these investigations should focus on and target the local adaptation to T3 availability.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Dutch cases and controls: Leiden University Medical Center; UK cases and controls: University of Oxford; Spanish cases and controls: Instituto Investigacion Sanitaria-Hospital Clinico Universitario Santiago; Greek cases and controls: University of Thessaly.

Provenance and peer review Not commissioned; externally peer reviewed.

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