CONCISE REPORT

Relationship between the functional exon 3 deleted growth hormone receptor polymorphism and symptomatic osteoarthritis in women


ABSTRACT

Background Several studies suggest a role of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis in the pathobiology of primary osteoarthritis (OA). A common polymorphism of the GH receptor (exon 3 deletion, d3-GHR) is associated with increased GH/IGF-1 activity.

Objective To study associations between the d3-GHR polymorphism and symptomatic OA.

Methods In the GARP (Genetics, osteoArthritis and Progression) study, we compared the d3-GHR polymorphism between OA patients and controls. GARP patients were genotyped for seven single nucleotide polymorphisms encompassing the d3-GHR gene, using rs4590183 as proxy for d3-GHR (pairwise $r^2=1$). Binary logistic regression models with robust SEs were performed, stratified by sex. For replication, rs4590183 was tested in three additional cohorts. Fixed- and random-effects combined analyses were performed.

Results In female GARP patients with severe familial OA, d3-GHR was associated with OA (adjusted OR 1.36 (95% CI 1.01 to 1.83), $p=0.043$), independently of age and body mass index. Combined analysis of all studies showed suggestive evidence for association between d3-GHR and OA (OR=1.17 (95% CI 1.04 to 1.30), $p=0.008$). Evidence was strongest in hip OA cases, without any evidence for heterogeneity.

Conclusions In women, the d3-GHR polymorphism was associated with symptomatic OA, especially at the hip site.

INTRODUCTION

Osteoarthritis (OA) is a debilitating disease characterised by progressive degradation of articular cartilage and bone remodelling. Although the exact pathogenesis remains to be elucidated, genetic studies have identified several variants associated with primary OA, such as the 7q22 containing multiple potential genes, growth differentiation factor 5 (GDF5) gene, frizzled related protein gene, deiodinase iodothyronine type II (DIO2) gene and mothers against decapentaplegic homologue 3 (SMAD3) gene. Together, these genes provide evidence that endochondral ossification may be involved in OA onset.

Endochondral ossification is driven by growth plate chondrocytes, resulting in longitudinal skeletal growth through a combination of proliferation, extracellular matrix (ECM) secretion and hypertrophy. Subsequently, terminally differentiated chondrocytes die and are replaced with bone tissue. At all stages, chondrocyte behaviour is tightly regulated by a complex network of interactions between circulating hormones, locally produced growth factors and ECM components. These chondrocytes likely play a role in bone shape and/or the quality of articular cartilage. One of the strongest stimulators of chondrocyte proliferation is growth hormone (GH), predominantly via insulin-like growth factor-1 (IGF-1) secretion. This qualifies variations within GH/IGF-1 genes as obvious candidates for association studies.

We hypothesise that the d3-GHR polymorphism increases OA risk by increasing (local) GH/IGF-1 activity. Therefore, we compared the effects of the d3-GHR polymorphism between cases with symptomatic generalised OA and controls. We tested for confirmation in three additional cohorts. A combined analysis was performed in women (2175 OA cases and 2623 controls).

PATIENTS AND METHODS

Subjects

The discovery study was the GARP (Genetics ARthrosis and Progression) study. For replication, the PAPIRAK (Patients Prospectively Recruited In Knee and hip Arthroplasty)/RAAK (Research Articular osteoArthritis Cartilage), ACRO (acromegaly) and Rotterdam studies were used. All patients and controls were from Dutch descent. Details of original study design and phenotype definition are described in online supplementary file 1.

Genotyping

Genomic DNA was isolated from peripheral blood according to standard procedures. In GARP, the
d3-GHR polymorphism was detected as described previously,\textsuperscript{12} based on specific amplification of the wild type (935 base pairs (bp)) and mutant (532 bp) alleles. To allow high-throughput genotyping, we assessed linkage disequilibrium (LD) between d3-GHR and single nucleotide polymorphisms (SNPs) covering the gene as determined in a GWAS, by means of Illumina 660W GWAS details are published elsewhere.\textsuperscript{13} Previously, only one SNP (rs6873545) was described to capture the d3-GHR polymorphism.\textsuperscript{14} We genotyped 373 GARP subjects and 752 controls for seven other SNPs (rs4590183, rs13354167, rs7721081, rs7701605, rs4242116, rs6878512, rs10941583), all being in high LD with the d3-GHR polymorphism (see online supplementary file 2). All SNPs were in Hardy–Weinberg equilibrium. In cases and controls, rs4590183 was selected as proxy for d3-GHR genotype ($r^2=1$). Throughout this report, the d3 allele of rs4590183 was designated as risk allele.

Replication cohorts: samples of the Rotterdam study were genotyped with the Illumina HumanHap 550v3 Genotyping BeadChip. GWAS details are published elsewhere.\textsuperscript{15} Other cohorts were genotyped by mass spectrometry using the homogeneous MassARRAY system of Sequenom (San Diego, California, USA) using standard conditions.

**Study design/statistical analysis**

First, association with d3-GHR with OA was performed in the GARP study (men and women) since this study consists of genetically enriched patients with symptomatic OA at multiple joint sites. Subsequently, we tested for confirmation in women of three other cohorts, the PAPRIKA/RAAK (joint replacement), acromegaly (signs of clinical and radiographic OA) and Rotterdam (severe radiographic OA) studies.

Logistic regression analyses were performed with STATX V10.1. A dominant genotypic model was applied. To adjust for family relationships within the GARP study, robust SEs were estimated from the variance between sibling pairs.\textsuperscript{16} Combined analyses were performed in women, using R V2.15.0.\textsuperscript{17} If the heterogeneity metric $I^2$ exceeded 25%, a random-effects model was also used, otherwise only a fixed-effects model was applied. Given that only one polymorphism was studied with well established functional effects, $p<0.05$ was considered as reflecting significance.

**RESULTS**

Table 1 shows the phenotypic characteristics of the GARP study (discovery sample). As shown in table 2, we found evidence for association between the d3-GHR polymorphism and OA, only in women of the GARP study (OR=1.36, 95% CI 1.01 to 1.83, $p=0.043$). Adjustment for age and body mass index (BMI) did not significantly affect the genotypic association.

Since women drove the association with d3-GHR, our replication was aimed at women with symptomatic OA of the PAPRIKA/RAAK and ACRO studies, and severe radiographic OA in the Rotterdam study (table 1). For the combined analysis, 2175 cases and 2623 controls were available, and the respective genotype frequencies are shown in online supplementary file 3. Although the association with d3-GHR was significant only in the PAPRIKA/RAAK study, the combined analysis of four studies with OA at any joint location provides evidence for association with d3-GHR, with an OR of 1.17 (95% CI 1.04 to 1.32, $p=0.008$), without any evidence for heterogeneity ($p=0.470$, $I^2=0\%$) (table 2). In a sensitivity analysis excluding the discovery GARP study, the association persisted (OR=1.14, 95% CI 1.01 to 1.30, $p=0.042$).

When we stratified for joint site in the combined analysis (table 2), we observed consistent effect sizes of approximately 1.2–1.3 among the joint strata, being significant in hip OA cases ($p=0.002$), without evidence for heterogeneity (figure 1B). Allelic data are presented in online supplementary file 4.

**DISCUSSION**

In a combined analysis of 2175 female OA cases and 2623 controls, we found evidence for association between the functional d3-GHR polymorphism and symptomatic OA (pooled OR=1.17, 95% CI 1.04 to 1.32, $p=0.008$), without evidence of heterogeneity and independently of age and BMI. Stratifying by joint site indicated that the association was most predominant in female cases with hip OA.

Human GH is a strong modulator of important physiological processes such as fuel homeostasis, cell differentiation and metabolic control. The GH/IGF-1 axis is essential for longitudinal skeletal growth. During growth, long bones increase in height through endochondral ossification, replacing a cartilage model by bone tissue. The main player in this process is the...
chondrocyte. GH is a main stimulator of chondrocyte proliferation in the growth plate, and, to a lesser extent, of ECM secretion and the hypertrophic switch of post-proliferative chondrocytes.\(^5\) Chondrocytes in OA cartilage share a fair amount of their expressed genes with those expressed in the terminal layer of the growth plate.\(^3\) Therefore, genes involved in skeletal morphogenesis early in life determining joint shape, might play a late-acting deleterious role towards OA. IGF-1 is associated with increased cartilage formation and laxity of peri-articular ligaments, and plays a role in osteophyte development.\(^18\) All these changes together contribute to an altered joint geometry, eventually resulting in an arthritic joint. The d3-GHR polymorphism is hypothesised to accelerate the OA process in susceptible patients by increasing GH responsiveness and, thereby, (local) IGF-1 levels.

Typically, the OR observed in the GARP discovery study was higher when compared to the replication studies but also generally higher than large scale GW A studies, such as that of Zeggini et al.\(^19\) This could be explained by the fact that for the GARP study we have applied a family-based sampling scheme towards the extreme spectrum of the OA phenotype, consisting of sibling pairs with both symptomatic and radiographic OA at multiple sites. In general, such a study is tailored to find genetic variants in the low frequency range with moderate to large effect sizes. Here, the GARP phenotype may have been most efficient in detecting predisposition of the d3-GHR

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**Table 2** Study-specific and combined analyses investigating the association between the d3-GHR polymorphism and symptomatic OA in women for OA at any joint site, and combined analyses after joint stratification

<table>
<thead>
<tr>
<th>Studies</th>
<th>OA at any joint site*</th>
<th>Heterogeneity †</th>
<th>Combined analyses*</th>
<th>Heterogeneity †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)‡</td>
<td>p Value</td>
<td>I²</td>
<td>p Value</td>
</tr>
<tr>
<td>GARP*</td>
<td>1.36 (1.01 to 1.83)</td>
<td>0.043</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PAPRIKA/RAAK*</td>
<td>1.32 (1.00 to 1.73)</td>
<td>0.048</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ACRO*</td>
<td>1.17 (0.73 to 1.90)</td>
<td>0.514</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RDAM*</td>
<td>1.09 (0.94 to 1.27)</td>
<td>0.244</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Combined analysis</td>
<td>1.17 (1.04 to 1.32)</td>
<td>0.008</td>
<td>0%</td>
<td>0.470</td>
</tr>
</tbody>
</table>

*Data were presented for female OA cases only.
†Heterogeneity across the OA studies quantified by the I² statistic, whereas its statistical significance was determined by the χ² distributed Cochran Q statistic.\(^20\)
‡OR (95% CI) and p values were calculated for the dominant genotypic model, and were presented with the minor allele (exon 3 deletion, d3) as the dominant risk allele. rs4590183 was used as proxy for the d3-GHR polymorphism in all cases and controls. The OR represents the risk of having the d3-GHR polymorphism in female OA cases versus controls, as shown for the total cohort and stratified for joint site. Logistic regression analysis is performed, applying a binary logistic model.

ACRO, acromegaly patients; GARP, Genetics ARthrosis and Progression study; NA, not applicable; OA, osteoarthritis; PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA).

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**Figure 1** Forest plots for the association between the d3-GHR polymorphism and symptomatic OA in women. Results are presented for the combined analyses of the GARP, PAPRIKA/RAAK, ACRO and Rotterdam studies, showing the association with OA at any joint site (top), and hip OA (bottom). GHR, growth hormone receptor; ROA, radiographic osteoarthritis; GARP, Genetics osteoARthritis and Progression study; PAPRIKA, PAtients Prospectively Recruited In Knee and hip Arthroplasty; RAAK, Research Articular osteoArthritis Cartilage; ACRO, acromegaly patients, ROTTERDAM, cases from the Rotterdam study.
polymorphism, although the allele frequency is not rare. Moreover, in a sensitivity analysis excluding GARE the association between the D3-GHR polymorphism and OA persisted, whereas the consistency of the effect sizes among the different cohorts and joint strata adds to the credibility of D3-GHR.

Several potential limitations need to be addressed. First, although the direction and effect sizes were similar in our replication cohorts, only the association in the PAPRIKA/RAAK cohort was significant. This is likely to be explained by the low number of cases in the ACRO and Rotterdam studies, providing insufficient power. Second, inclusion of acromegaly patients might introduce a bias, since disease processes in acromegalic arthropathy may differ from those in primary OA. However, since the D3-GHR polymorphism does not predispose to acromegaly itself, the inclusion of acromegalics with OA is not likely to influence our results. Merely, we expect that a general detrimental effect of GH excess on joint tissue homeostasis predisposes to OA. Finally, the unknown OA status in controls might have led to an underestimation of the reported effect.

In conclusion, we found an association between the D3-GHR polymorphism and symptomatic OA in women, especially in cases with hip OA. Being aware of the tendency of association studies to produce false-positive results, additional replication is necessary. Furthermore, studying the D3-GHR polymorphism in relation to GH profiles and IGF-1 levels could further elucidate the role of the GH/IGF-1 axis in OA.

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Contributors

All authors contributed to the conception and design, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and gave final approval of the version to be published.

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Competing interests

None.

Patient consent

Patient informed consent form of the respective studies (Leiden University Medical Center, Erasmus Medical Center Rotterdam, The Netherlands). All patients gave informed consent.

Ethics approval

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REFERENCES

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