Abstract

Objective. The identified osteoarthritis (OA) susceptibility genes are mainly active in skeletal development and could thus affect joint geometry. Because nonoptimal joint geometry is a risk factor for the development of OA, we investigated if and how the path that leads from nonoptimal joint geometry to OA of the hip is influenced by these genes.

Methods. The shape of the hips of subjects in the Genetics, Osteoarthritis and Progression Study, consisting of sibling pairs with symptomatic OA at multiple joint locations, was quantified by applying a statistical shape model to radiographs. Shape aspects (modes) were correlated to OA characteristics. We then tested for the association of shape modes with OA susceptibility single-nucleotide polymorphisms (SNPs) of GDF5, FRZB, and DIO2.

Results. Four of 23 shape modes (mode 1, mode 17, mode 18, and mode 21) were strongly associated with OA characteristics. We observed a significant interaction between carrier status of DIO2 rs12885300 and hip OA characteristics for mode 1 ($P = 0.005$). This indicates that this specific aspect of hip shape correlates with OA characteristics only in carriers of the susceptibility allele.

Conclusion. Our results suggest that it is more likely that the rs12885300 SNP of DIO2 increases the vulnerability of cartilage to nonoptimal bone shapes rather than directly influencing the formation of these shapes.

The genetic predisposition for osteoarthritis (OA) (1) may reflect the result of multiple interacting genes with small effects (2). The identification of such genes should be considered essential to obtain a better understanding of OA and the underlying biologic events preceding its onset (3). One path leading to OA in which genes might play a role starts with the causal effect of hip morphology on the development of OA and may explain part of the heritability of hip OA. The life-long biomechanical stress caused by a nonoptimal shape of the bones in the joint leads to recurrent damage of cartilage, which eventually triggers the onset of OA (4).

What is interesting in this respect is the fact that genes that are mainly active in skeletal development, such as FRZB and GDF5, are identified as OA susceptibility genes (5,6). These genes are involved in the orchestration of growth plate chondrocytes that results in formation of cartilage and eventually bone during endochondral ossification (7). A recent large-scale meta-analysis (6) did not provide evidence for the relatively rare FRZB OA risk alleles, indicating that these variants do not confer susceptibility to common OA. It is, however, generally known that meta-analyses have little power to detect relatively rare variants with a modest effect that confer risk in a specific subset of OA cases. Furthermore, functional studies in Frzb-knockout mice showed increased cortical bone thickness and density, resulting in stiffer bones upon mechanical loading, which may increase OA susceptibility and stresses developmental aspects (8).

In the Genetics, Osteoarthritis and Progression...
(GARP) Study, our group identified, using a genome-wide linkage approach, the deiodinase, iodothyronine, type II (D2) gene \((DIO2)\) as an important susceptibility gene \((9)\). \(DIO2\) encodes D2, an enzyme that, together with its counterpart deiodinase 3 (D3), regulates the intracellular bioavailability of the active thyroid hormone T3. In the growth plate, T3 initiates the terminal differentiation of hypertrophic chondrocytes, which is important for the subsequent formation of long bones. These results suggest that the process of chondrogenesis may be an important factor underlying the etiology of OA onset and/or progression.

In the current study, we investigated whether OA susceptibility genes that are involved in endochondral ossification influence the effect of joint shape on OA. These genes might either affect the shape itself or moderate how the shape affects the cartilage. The aims of the study were to investigate the correlation between hip shape and OA characteristics and to investigate whether the OA susceptibility alleles of \(FRZB\), \(GDF5\), and \(DIO2\) explain a part of the relationship between OA and aspects of shape.

**PATIENTS AND METHODS**

The **GARP Study.** The data presented here are part of the ongoing GARP Study, which comprises 190 Caucasian sibling pairs and 2 trios of Dutch ancestry, in which the proband is affected with symptomatic OA at multiple sites. The details of patient ascertainment and assessment of OA characteristics have been previously described \((1)\). For the current study, cross-sectional data from the baseline visit were used. Hips with joint replacements (23 unilateral and 15 bilateral) were excluded. The GARP Study was approved by the Medical Ethics Committee of the Leiden University Medical Center.

**Statistical shape models (SSMs).** An SSM allows the quantitative description of the total shape of an object and all possible variation that exists in a study population. We created an SSM of the shape of the hip in anteroposterior radiographs, using the freely available Active Shape Model toolkit (Manchester University, Manchester, UK) \((10)\). The model consisted of 70 landmark points on contours along the femur, acetabulum, and pelvis. The lateral side of the femur was omitted from the model due to poor visibility on many radiographs. Images of the left side were mirrored so they could be entered into the model as right hips.

After annotating the images, all contours were overlaid using the mathematical center, and all contours were scaled and rotated to obtain an optimal fit. Finally, we used principal components analysis to recombine the position of the contour points into shape modes (the principal components). These modes thus reflect how variation in the position of the points is correlated and describe specific patterns of variation in shape that exist within the study population. We retained enough modes to explain 95% of the variation in the appearance of the study population.

![Figure 1](image-url). Visual representation of the shape modes that were strongly associated with radiographic hip osteoarthritis (OA), as defined by Kellgren/Lawrence grade \(\geq 2\). Each mode represents a specific pattern in the variation in shape, in which the left and right images show opposite extremes of that specific pattern. Because the modes with higher numbers represent more subtle variations in shape, the images represent exaggerated extremes, as indicated by the standard deviation. Arrows indicate details that vary between the extremes. Dotted lines represent visual aids to appreciate variation in width.

**Statistical analysis.** We used generalized estimating equations \((11)\) for the association analyses between the shape modes and hip OA features and genotypes, as implemented in
SPSS version 16.0. We adjusted for the familial dependencies of the sibling pairs and the correlation between the left and right hip joints by including a $4 \times 4$ correlation matrix in the regression, in which all coefficients could vary independently.

To estimate heritability of hip shape, we fitted linear regression models to the shape data with 2 Gaussian-distributed random effects, namely one family effect that is shared by all 4 hips of a sibling pair and an individual effect that is shared by the 2 hips of a sibling. By using the variance of the familial, person, and residual components, we estimated the correlation of the shape modes within a person and between sibling pairs. Instead of adjusting $P$ values a priori for multiple testing, nominal $P$ values are provided in order to allow interpretation of the level of significance.

### RESULTS

**Characteristics of the study population.** The study group comprised 341 of 386 subjects for whom radiographs of the left and/or right side that were of sufficient quality were available ($n = 656$ hips). Although all subjects in the GARP cohort have symptomatic OA at multiple joint locations, only 93 (14%) of the 656 hips had radiographic hip OA (Kellgren/Lawrence [K/L] grade $\geq 2$) (12) of the left and/or right side, which allowed us to investigate shape aspects related to the hip OA phenotype and compare these with those of family members without hip OA. The average age of the subjects was 59.9 years (range 43–79 years), the average body mass index (BMI) was 27.1 kg/m$^2$ (range 19–46), and 18% were men. The shape model consisted of 23 principal modes of shape variation. The beginning modes represent the large, overall patterns in shape variation that exist in the GARP population, while the latter modes represent very subtle variations in shape.

**Hip shape and OA.** After adjustment for age, sex, and BMI, 4 modes (M1, M17, M18, and M21) showed a highly significant association with radiographic characteristics of hip OA as defined by the K/L score (for M1, $P = 0.006$; for M17, $P = 5 \times 10^{-5}$; for M18, $P = 0.002$; for M21, $P = 2 \times 10^{-5}$). These modes represent various distinct aspects of femoral and pelvic morphology, as shown in Figure 1.

Mode 1 represents a lateral rotation of the femur with respect to the pelvis. Patients with OA have a large angle between the femur and the pelvis. Given the use of a positioning frame that fixed the position of the feet during radiography, this mode must represent variation in pelvic width. Mode 17 represents a combined but opposite rotation of the femur and the pelvis around the vertical axis; patients with OA have a more internally rotated femur combined with a backward-rotated pelvis. Mode 18 represents a variation in the acetabular version angle, in which the femur is positioned corresponding to the acetabular version. In patients with OA, the acetabulum faces the frontal plane, and the femur is rotated internally. Mode 21 shows very subtle variations in the size of both trochanters and in the depth of the acetabulum. Patients with OA have larger trochanters, a bulky head, and a deep acetabulum.

### Table 1. Heritability estimation of the selected shape modes, in subjects with osteoarthritis (OA) and subjects without OA*

<table>
<thead>
<tr>
<th>Mode</th>
<th>Variance component*</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Familial</td>
<td>Person</td>
</tr>
<tr>
<td>Subjects with OA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>0.23 (0.02–2.92)</td>
<td>0.65 (0.25–1.67)</td>
</tr>
<tr>
<td>M17</td>
<td>0.38 (0.11–1.28)</td>
<td>0.05 (0.00–2.056)</td>
</tr>
<tr>
<td>M18</td>
<td>0.18 (0.03–1.09)</td>
<td>0.20 (0.04–1.12)</td>
</tr>
<tr>
<td>M21</td>
<td>0</td>
<td>0.43 (0.24–0.76)</td>
</tr>
<tr>
<td>Subjects without OA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>0.23 (0.11–0.46)</td>
<td>0.48 (0.34–0.67)</td>
</tr>
<tr>
<td>M17</td>
<td>0.07 (0.01–0.41)</td>
<td>0.34 (0.21–0.55)</td>
</tr>
<tr>
<td>M18</td>
<td>0.17 (0.07–0.39)</td>
<td>0.33 (0.21–0.54)</td>
</tr>
<tr>
<td>M21</td>
<td>0.12 (0.04–0.40)</td>
<td>0.38 (0.24–0.6)</td>
</tr>
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</table>

* Values are the heritability estimate (95% confidence interval).

Effect of OA risk alleles on the relationship between hip shape and OA characteristics. To investigate if and how OA susceptibility genes influence the relationship between shape modes and OA, we first looked at the association between the previously identified OA susceptibility alleles of $FRZB$, $GDF5$, and $DIO2$ and the shape modes M1, M17, M18, and M21, which...
were associated with hip OA characteristics. This analysis corresponded to the hypothesis that these genes play a role in the formation of OA-causing joint shapes. However, we could not detect such an association, neither when all subjects were included nor when the subjects without OA were studied separately (results not shown).

Next, we investigated whether the relationship between these hip shape modes and OA was different between carriers and noncarriers of the OA risk alleles, by testing the interaction of gene carrier status and hip OA characteristics. This analysis corresponded to the notion that OA susceptibility genes affect the cartilage and thus increase the vulnerability to biomechanically compromising shapes. As shown in Figure 2, we observed an interaction among carriers of the DIO2 rs12885300 allele and hip OA characteristics for M1 and M18, which was significant only for M1 (P = 0.005). An opposite interaction between carriers of the DIO2 rs12885300 allele and hip OA characteristics was observed for M21 (P = 0.02) (Figure 2). We did not observe any interaction between carrier status for the GDF5 or FRZB single-nucleotide polymorphisms (SNPs) and hip OA characteristics with shape modes.

**Discussion**

Recent advances in genome-wide searches in OA launched the tantalizing hypothesis that the process of skeletal morphogenesis, regulated by identified OA susceptibility genes, may lead to deviations in hip shape that are an important risk factor for late-onset OA (13). In the current study, we investigated if and how OA susceptibility SNPs of DIO2, GDF5, and FRZB influence the path that leads from hip shape to OA. We were able to identify several aspects of hip morphology that were strongly correlated with the presence of hip OA characteristics.

We investigated the heritability of hip shape by calculating the correlation of the identified OA shape
modes between sibling pairs and within a person, stratified by hip OA status. Shape modes 1 and 18 showed a relatively high within-person correlation that appeared to be independent of the presence of OA characteristics, which may indicate that these shape aspects are innate and precede the onset of OA. This view is supported by the nature of these shape aspects, because the width of the pelvis and the acetabular version are not likely influenced by the OA process itself. In contrast, for mode 17, we observed a relatively high between–sibling pair correlation only in the presence of OA in the respective hip, indicating that this mode may be the result of, or monitors, the OA process. This could possibly represent adaptation of the standing position, perhaps due to limitations in function or due to pain as a result of similar OA pathophysiology. Given the relatively small numbers of sibling pairs that shared hip OA, these aspects should be further investigated in a follow-up study.

Next, we investigated if and how the OA susceptibility genes are involved in the identified relationship between hip shape and OA characteristics, by testing for interaction between the SNP carrier status and hip OA characteristics. Such an interaction would indicate that the relationship between hip shape and OA depends on carrier status.

We observed interactions between DIO2 SNP rs12885300 and hip OA characteristics for mode 1 and mode 21 (Figure 2). The interaction term for mode 1 yielded a \( P \) value of 0.005, which could be considered significant after Bonferroni correction when <10 tests would have been done. Due to dependence between the tests, it is difficult to provide a correction factor. Because we tested 5 SNPs of 3 genes (DIO2, GDF5, and FRZB), the correction factor should be somewhere between 4 and 20; thus, replication is needed to confirm this result. If the result is indeed true, it indicates that a narrow hip (mode 1) is a risk for OA, especially in carriers of DIO2 SNP rs12885300 OA risk alleles. A likely explanation could be that the OA susceptibility SNPs affect cartilage structure or metabolism such that the cartilage becomes more vulnerable to the biomechanical stress caused by nonoptimal hip morphology.

The interaction between OA and shape mode 21 (in which patients with OA appear to have a deeper acetabulum) with the DIO2 SNPs is more difficult to understand, because the interaction term is reversed: the association is actually not present for carriers of the OA susceptibility alleles. It is possible that although DIO2 SNP rs12885300 affects cartilage such that it is more resistant against one type of stress, the cartilage could simultaneously become more vulnerable for another type of stress (e.g., compression versus shear stresses). However, given the modest significance \( (P = 0.02) \), this finding might very well be a false-positive result and stresses the need for replication of our findings in an additional data set.

Previously, we showed that a DIO2 haplotype consisting of the minor allele of SNP rs225014 and the major allele of SNP rs12885300 showed the most consistent association with hip OA in women in subsequent replication by association across different OA studies (9). In the current study, the observed interaction between mode 1 and OA with the common (OA risk) allele of SNP rs12885300 is also apparent for the rare (OA risk) allele of SNP rs225014; however, it is less explicit, and the interaction term appeared to be not significant (results not shown). This could be attributable to the lower number of carriers of the risk allele with OA (power), due to the fact that the functional variant has higher linkage disequilibrium with the major allele rs12885300 or genetic heterogeneity.

Strictly speaking, because this is a cross-sectional study, the above-described interactions could also be interpreted as follows: that the ongoing OA process affects hip morphology differently depending on carrier status. However, considering the overall nature of these aspects of morphology (e.g., mode 1 describes the width of the hips, while mode 18 describes the acetabular version), it is likely that these aspects precede OA and are thus a risk factor for OA development and progression, depending on carrier status. Because stratification (especially for the less heterozygous GDF5 SNP) consumes sample size and power, these analyses should be replicated in a larger data set.

Because DIO2, GDF5, and FRZB were indicated to be involved in both OA and the endochondral ossification process and thus in bone morphogenesis, we initially expected to observe a direct association between the shape modes that were associated with OA characteristics and the OA susceptibility SNPs. However, we did not observe such an association that would have supported the idea that the OA susceptibility genes affect the morphogenesis, resulting in nonoptimal shapes that cause the onset of OA. However, we did observe direct associations between DIO2 SNP rs12885300 and GDF5 SNP rs143383 and 2 other shape modes that were not associated with OA characteristics (modes not shown). These modes represent different characteristics of hip morphology such as “slenderness” of the hip (in the case of the GDF5 SNP) and acetabular geometry (in the case of the DIO2 SNP). This indicates...
that these genes may play a measurable role in morphogenesis of the hip joint.

We established in-depth phenotyping by assessing subtle shape aspects of the hip joints within subjects of the GARP Study who had symptomatic OA at multiple joint locations. Further and larger studies are necessary to assess robust genetic variants that determine these shape aspects of hip joints, preferably via genome-wide approaches.

Among carriers of some of the DIO2 OA susceptibility alleles, we observed an interaction between carrier status and the association between hip morphology and OA. Although these findings require replication, they suggest that it is more likely that OA risk alleles of DIO2 increase the vulnerability of cartilage for nonoptimal bone shapes rather than directly influencing the formation of these shapes.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Waarsing had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Waarsing, Kloppenburg, Slagboom, Weinans, Meulenbelt.

**Acquisition of data.** Waarsing, Kloppenburg, Kroon, Meulenbelt.

**Analysis and interpretation of data.** Waarsing, Kloppenburg, Kroon, Houwing-Duistermaat, Weinans, Meulenbelt.

**REFERENCES**