IX.

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

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ABSTRACT

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

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 7 single nucleotide polymorphisms encompassing the d3-GHR gene, using rs4590183 as proxy for d3-GHR (pairwise r2=1). Binary logistic regression models with robust standard errors 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 odds ratio (OR) 1.36 (95%CI 1.01-1.83), p=0.043), independently of age and BMI. Combined analysis of all studies showed suggestive evidence for association between d3-GHR and OA (OR=1.17 (95%CI 1.04-1.30), 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 characterized by progressive degradation of articular cartilage and bone remodelling (1). 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 (FRZB) gene, Deiodinase IOdothyronine type II (DIO2) gene and Mothers Against Decapentaplegic homolog 3 (SMAD3) gene (2). Together, these genes provide evidence that endochondral ossification may be involved in OA onset (3).

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 (4). 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 (5). This qualifies variations within GH/IGF-1 genes as obvious candidates for association studies.

The dimeric GH receptor (GHR) mediates the effect of GH on target tissues and exists of different molecular structures, depending on assortments of coding polymorphisms in the GHR gene. Three GHR variants, differing in the presence or absence of exon 3 (GHRfl-fl, GHRfl-d3, GHRd3-d3), are commonly seen. Exon 3 loss (d3-GHR) results in a truncated receptor with increased GH responsiveness by enhanced signal transduction (6;7).

We hypothesize 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 generalized 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 (8). For replication, the PAPRIKA (PAtients Prospectively Recruited In Knee and hip Arthroplasty)/RAAK (Research Articular osteoArthritis Cartilage), ACRO (acromegaly) and Rotterdam Studies were used (9-11). All patients and controls were from Dutch descent. Details of original study design and phenotype definition were described in *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 (12), based on specific amplification of the wild type (935 base pairs (bp)) and mutant (532bp) 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 (13). Previously, only one SNP (rs6873545) was described to capture the d3-GHR polymorphism (14). We genotyped 373 GARP subjects and 752 controls for 7 other SNPs (rs4590183, rs13354167, rs7721081, rs7701605, rs4242116, rs6878512, rs10941583), all being in high LD with the d3-GHR polymorphism (*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 (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 STATA Statistical Software version 10.1 (Statacorp, College Station, TX). A dominant genotypic model was applied. To adjust for family relationships within

the GARP Study, robust standard errors were estimated from the variance between sibling pairs (16). Combined analyses were performed in women, using R version 2.15.0(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 describes 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 1.01-1.83, 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 *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 1.04-1.32,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 1.01-1.30, 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 2B*). Allelic data were presented in *Supplementary File 4*.

Table 1. Clinical characteristics of the discovery and replication studies

	Discovery study				Replication studies					
Clinical characteristics	**GARP Females (N = 305)**	**Males (N = 68)**	**Controls Females (N = 435)**	**Males (N = 317)**	**PAPRIKA/RAAK Females (N = 332)**	**Controls Females (N = 563)**	**ACRO Females (N = 41)**	**Controls Females (N = 361)**	**Rotterdam Females (N = 1497)**	**Controls Females (N = 1264)**
Age, years	59.8 (7.6)	61.3 (7.6)	57.0 (6.9)	61.3 (7.2)	61.9 (6.5)	57.7 (1.4)	60.8 (12.2)	57.7 (1.4)	70.0 (8.1)	66.3 (7.8)
BMI, kg/m²	27.0 (4.9)	27.1 (3.4)	25.4 (3.9)	25.8 (3.2)	NR	NR	28.4 (4.9)	NR	27.1 (4.1)	26.2 (3.9)
ROA, by joint site*										
Knee (n (%))	116 (38%)	26 (38%)	NA	NA	121 (36%)	NA	18 (44%)	NA	284 (19%)	NA
Hip (n (%))	76 (25%)	19 (28%)	NA	NA	211 (64%)	NA	12 (29%)	NA	246 (16%)	NA
Hand (n (%))	261 (86%)	61 (90%)	NA	NA	NA	NA	32 (78%)	NA	1310 (88%)	NA
ROA definition	KL≥2**	KL≥2**	NA	NA	THP/TKP***	NA	KL≥2**	NA	KL≥3**	NA

Data are shown as mean (SD), unless mentioned otherwise. For the replication studies, only female data are shown.

GARP, Genetics osteoARthritis and Progression Study (primary OA); PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA); ACRO, acromegaly patients; Rotterdam, cases of the Rotterdam Study; BMI, body mass index; ROA, radiographic osteoarthritis; KL, Kellgren-Lawrence; THP, total hip prosthesis; TKP, total knee prosthesis; NA, not applicable. NR, not reported.
**, In the GARP Study, 8 patients had a unilateral knee prosthesis, 1 patient had bilateral knee prostheses, 22 patients had a unilateral hip prosthesis and 15 patients had bilateral hip prostheses, due to end-stage OA. All cases with prostheses were from separate families.*
***, Hand OA was defined as KL≥2 in 2 of the following 3 groups: distal interphalangeal (DIP), proximal interphalangeal (PIP), carpometacarpal (CMC) or trapezium scaphoid (TS) joints.*
****, 98% of patients had Kellgren Lawrence ≥2 pre-operatively*

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

Studies	OA at any joint site*		Heterogeneity***		joint site	Combined analyses*		Heterogeneity***	
	OR (95%CI)**	p-value	I^2	p-value		OR (95%CI)**	p-value	I^2	p-value
GARP*	1.36 (1.01-1.83)	0.043	NA	NA	**Hip OA**	1.34 (1.11-1.62)	0.002	0%	0.709
PAPRIKA / RAAK*	1.32 (1.00-1.73)	0.048	NA	NA	**Knee OA**	1.20 (0.84-1.72)	0.308	66.7%	0.029
ACRO*	1.17 (0.73-1.90)	0.514	NA	NA	**Hand OA**	1.29 (0.99-1.67)	0.055	51.7%	0.126
RDAM*	1.09 (0.94-1.27)	0.244	NA	NA					
Combined analysis	1.17 (1.04-1.32)	0.008	0%	0.470					

**, Data were presented for female OA cases only.*
***, 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 vs controls, as shown for the total cohort and stratified for joint site. Logistic regression analysis is performed, applying a binary logistic model.*
****, Heterogeneity across the OA studies quantified by the I2 statistic, whereas its statistical significance was determined by the X2 distributed Cochran Q statistic (20).*

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

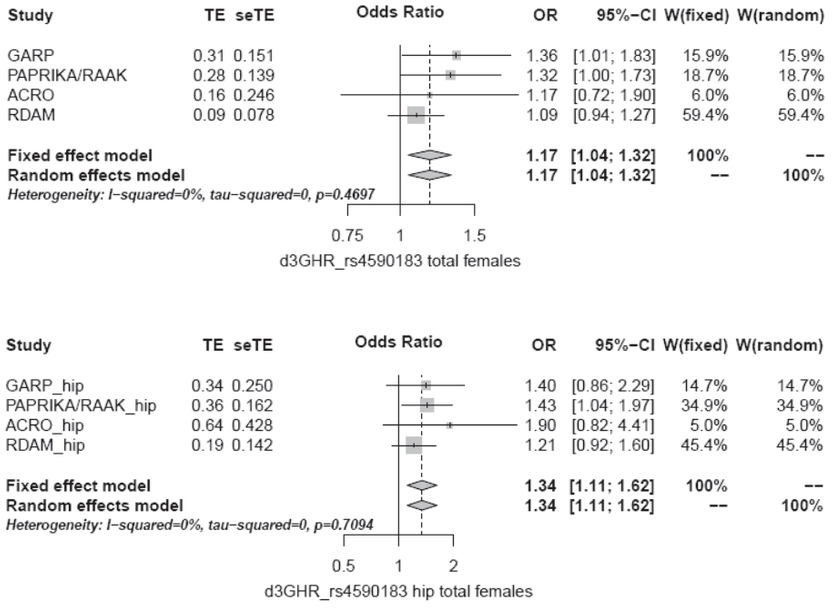

Figure 1. Forest plots for the association between the d3-GHR polymorphism and symptomatic OA in females. Results are presented for the combined analyses of the GARP, PAPRIKA/RAAK, ACRO and Rotterdam Studies, showing the association with OA at any joint site (A), and hip OA (B).

GHR, growth hormone receptor; ROA, radiographic osteoarthritis; GARP, Genetics osteoARthritis and Progression Study; PAPRIKA, PAPRIKA, PAtients Prospectively Recruited In Knee and hip Arthroplasty; RAAK, Research Articular osteoArthritis Cartilage; ACRO, acromegaly patients, ROTTERDAM, cases from the Rotterdam Study.

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 1.04-1.32, 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 hypothesized 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 GWA studies, such as 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 polymorphism, although the allele frequency is not rare. Moreover, in a sensitivity analysis excluding GARP, 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. Firstly, 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 of ACRO and Rotterdam Study, providing insufficient power. Secondly, 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 is not predisposing 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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SUPPLEMENTARY FILE 1.

Study populations & Phenotype definition

The discovery study is the GARP (Genetics, osteoARthritis and Progression) Study, which is a prospective cohort study aimed at identifying determinants of OA susceptibility and progression. This study consists of 191 sibling pairs (N=382) affected by symptomatic and radiographic OA at multiple joint sites. Radiographic OA of the knee, hip and hands was assessed on conventional radiographs, according to the Kellgren-Lawrence (KL) scale (1;2). Radiographic knee and hip OA were defined by the ROA score (ROA ≥1) (3), in which the specific ROA score (0-2) represents no, unilateral or bilateral involvement based on KL ≥2. For hand ROA we used the following definition to be comparable to the definitions used in the Rotterdam Study (*vide infra*): KL≥2 in at least 2 of the following 3 groups: distal interphalangeal (DIP), proximal interphalangeal (PIP), carpometacarpal (CMC) or trapezium scaphoid (TS) joints (4). Details of patient recruitment and phenotypic description are reported in Riyazi *et al.* (2). We used data from the baseline visit, excluding one patient for unsuccessful DNA analysis and eight for missing radiographs, resulting in the inclusion of 373 patients. The GARP Study was compared with 753 independent individuals (*i.e.* partners) from the Leiden Longevity Study (5). One patient was excluded for missing data on sex, resulting in 752 controls.

In the confirmation and replication studies, only female cases and controls were studied. The PAPRIKA (PAtients Prospectively Recruited In Knee and hip Arthroplasty) and RAAK (Research Articular osteoArthritis Cartilage) cohorts consist of patients with total knee prosthesis (TKP) or total hip prosthesis (THP) for end-stage OA (98% had a pre-operative KL score of ≥2), and were, therefore, likely to have significant symptomatic disease. Female patients aged ≤70 years were selected with a TKP or THP for primary OA (N=332). Controls comprise a random selection of unrelated female subjects (N=563) from the Rotterdam Study (6), that were not selected on the basis of phenotype.

The acromegaly cohort consists of long-term well-controlled patients (7), of which 41 female patients were successfully genotyped and therefore included. All patients had signs of arthropathy at any joint. In the acromegalics, radiographic OA was defined by the ROA score, based on KL ≥2. The controls comprise a random selection of another sample of unrelated subjects (N=361) from the Rotterdam Study (6), that were not selected on the basis of phenotype.

The Rotterdam Study, which comprises 7983 Caucasian participants, is a prospective, population-based cohort study of determinants and the prognosis of chronic diseases in the elderly (6). Radiographs of the knee, hip and hands were made; however, symptomatic data were not available. In order to select comparable female cases for the GARP and PAPRIKA/RAAK Studies, and in the absence of symptomatic data, 1497 Rotterdam cases were selected according to the following definitions: knee and hip OA as KL ≥3, and hand OA as KL ≥2 in at least 2 of the following 3 groups (DIP, PIP, CMC/TS joints). Subjects did not show overlap to the Rotterdam control subjects mentioned above. Rotterdam cases were compared to 1264 other female controls from the Rotterdam Study.

All patients and controls were from Dutch descent. Study protocols were approved by the local Medical Ethics Committee and written informed consent was obtained from all participants.

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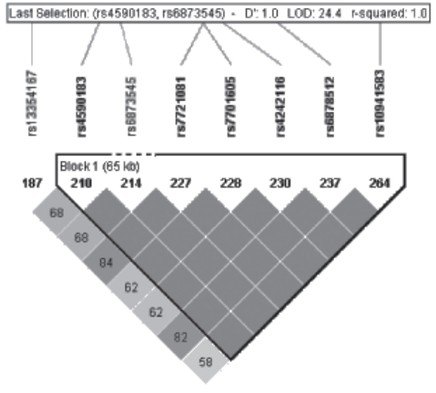
SUPPLEMENTARY FILE 2

Linkage disequilibrium patterns between the 7 SNPs selected from HAPMAP and the d3-GHR polymorphism.



SNP, single nucleotide polymorphism; GHR, growth hormone receptor.

SUPPLEMENTARY FILE 3.

Genotype frequencies of the d3-GHR polymorphism (using rs4590183) in the different case and control populations, for, respectively, the total cohort and female subjects only

Total cohort	**GARP**	**Controls**	**PAPRIKA/**	**Controls**	**ACRO**	**Controls**	**ROTTERDAM**	**Controls**
rs4590183	**(N = 373)**	**(N = 752)**	**RAAK**	**(N = 1000)**	**(N = 85)**	**(N = 668)**	**(N = 2198)**	**(N = 2645)**
N (freq)			**(N = 502)**					
GHR_{fl-fl}	202 (0.542)	442 (0.588)	268 (0.534)	579 (0.579)	50 (0.588)	374 (0.588)	1264 (0.575)	1585 (0.599)
GHR_{fl-d3}	154 (0.413)	260 (0.346)	206 (0.410)	354 (0.354)	29 (0.341)	255 (0.382)	817 (0.372)	936 (0.354)
GHR_{d3-d3}	17 (0.046)	50 (0.066)	28 (0.056)	67 (0.067)	6 (0.071)	39 (0.058)	117 (0.053)	124 (0.047)
Female subjects only	GARP	Controls	PAPRIKA/RAAK	Controls	ACRO	Controls	ROTTERDAM	Controls
rs4590183	(N = 305)	(N = 435)	(N = 332)	(N = 563)	(N = 41)	(N = 361)	(N = 1497)	(N = 1264)
N (freq)								
GHR_{fl-fl}	163 (0.534)	265 (0.609)	172 (0.518)	330 (0.586)	22 (0.537)	208 (0.576)	853 (0.570)	757 (0.599)
GHR_{fl-d3}	127 (0.416)	141 (0.324)	138 (0.416)	201 (0.357)	17 (0.415)	132 (0.366)	565 (0.377)	453 (0.358)
GHR_{d3-d3}	15 (0.049)	29 (0.067)	22 (0.066)	32 (0.057)	2 (0.049)	21 (0.058)	79 (0.053)	54 (0.043)

[a] *Females only.*

Data are shown as number (frequency). N, number of patients. GARP, Genetics osteoARthritis and Progression Study; PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/ Research Articular osteoArthritis Cartilage; ACRO, acromegaly patients; ROTTERDAM, cases of the Rotterdam Study.

SUPPLEMENTARY FILE 4A.

Study-specific and combined analyses by allele frequency data of rs4590183 for female cases with symptomatic OA

	GARP*		**PAPRIKA / RAAK***		**ACRO***		**RDAM***	
Joint site	OR (95%CI)**	p-value	OR (95%CI)**	p-value	OR (95%CI)**	p-value	OR (95%CI)**	p-value
Overall	1.17 (0.92-1.49)	0.205	1.28 (1.03-1.60)	0.025	1.08 (0.64-1.83)	0.763	1.07 (0.95-1.22)	0.280
Hip OA	1.29 (0.87-1.90)	0.203	1.31 (1.02-1.68)	0.038	1.30 (0.53-3.18)	0.569	1.17 (0.94-1.46)	0.160
Knee OA	1.40 (1.01-1.93)	0.042	1.24 (0.91-1.70)	0.172	1.05 (0.48-2.28)	0.930	0.87 (0.70-1.09)	0.230
Hand OA	1.30 (1.00-1.71)	0.052	NA	NA	1.23 (0.70-2.18)	0.473	1.09 (0.96-1.24)	0.190

	Combined analyses*		**Heterogeneity**	
Joint site	OR (95%CI)**	p-value	I^{2***}	p-value
Overall	1.13 (1.02-1.24)	0.016	0%	0.544
Hip OA	1.24 (1.06-1.44)	0.006	0%	0.924
Knee OA	1.11 (0.86-1.45)	0.414	56.2%	0.077
Hand OA	1.13 (1.01-1.27)	0.033	0%	0.472

**, Data were presented for female OA cases only.*
***, OR (95%CI) and p-values were calculated using the allelic model, and were presented with the minor allele (exon 3 deletion, d3) as risk allele.*
****, Heterogeneity across the OA studies quantified by the I2 statistic, whereas its statistical significance was determined by the X2 distributed Cochran Q statistic.*

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

SUPPLEMENTARY FILE 4B.

Frequencies of the minor allele (exon 3 deletion, d3) across the different study cohorts with patients with symptomatic OA and corresponding controls

Joint site	GARP*	PAPRIKA / RAAK*	ACRO*	RDAM* #
Overall	0.257	0.283	0.256	0.245
Hip OA	0.276	0.287	0.292	0.262
Knee OA	0.293	0.277	0.250	0.210
Hand OA	0.278	NA	0.281	0.248
Controls	0.229**	0.235***	0.241***	0.226***

**, Minor allele frequencies (MAF), with the exon 3 deletion (d3) allele as minor allele, are presented for the different studies, stratified by joint site with symptomatic OA.*
***, Controls of the Leiden Longevity Study.*
****, Controls of the Rotterdam Study.*

#, The Rotterdam Study did not comprise cases with symptomatic OA. We chose a severe radiographic phenotype (see Method section) in order to be comparable to the other cohorts with symptomatic OA.

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