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GENOME-WIDE ASSOCIATION AND FUNCTIONAL STUDIES IDENTIFY A ROLE FOR MATRIX-GLA PROTEIN IN OSTEOARTHRITIS OF THE HAND

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ABSTRACT

Objective Osteoarthritis (OA) is the most common form of arthritis and the leading cause of disability in the elderly. Of all the joints, genetic predisposition is strongest for OA of the hand, however only few genetic risk loci for hand OA have been identified. Our aim was to identify novel genes associated with hand OA and examine the underlying mechanism.

Methods We performed a genome-wide association study of a quantitative measure of hand OA in 12,784 individuals (discovery: 8,743, replication: 4,011). Genome-wide significant signals were followed up by analysing gene and allele specific expression in a RNA-sequencing dataset (n=96) of human articular cartilage

Results We found two significantly associated loci in the discovery set: at chr12 ($P=3.5 \times 10^{-10}$) near the MGP gene and at chr12 ($P=6.1 \times 10^{-9}$) near the CCDC91 gene. The DNA variant near the MGP-gene was validated in three additional studies, which resulted in a highly significant association between the MGP-variant and hand OA (rs4764133, $\text{Beta}_{\text{meta}}=0.83$, $P_{\text{meta}}=1.8 \times 10^{-15}$). This variant is high linkage disequilibrium with a coding variant in *Matrix Gla-Protein (MGP)*, a vitamin K-dependent inhibitor of cartilage calcification. Using RNA-sequencing data from human primary cartilage tissue (n=96), we observed that the hand OA MGP-risk allele was significantly lower expressed compared to the reference allele (40.7%, $P<5 \times 10^{-16}$).

Conclusions Our results indicate that the association between the MGP-variant and increased risk for hand OA is caused by a lower expression of *MGP*, which may increase the burden of hand OA by decreased inhibition of cartilage calcification.

INTRODUCTION

Osteoarthritis (OA) is the most frequent joint disorder worldwide. An estimated 22% of the adult population has a joint affected by OA and this incidence increases to 49% in individuals over 65 years of age (1). All synovial joints can be affected by OA, with hand OA as one of the most common forms of OA. Hand OA is characterized by osteophyte formation, bony enlargements of finger joints and cartilage degradation in the joints. One of the factors contributing to cartilage degradation is the increase of calcified cartilage in the joint (2,3). In addition, hand OA is related to the occurrence of OA at other sites, most notably with knee OA (4,5). Patients affected by hand OA suffer from pain and disability, impacting their quality of life. OA is a leading cause of chronic disability (6), yet currently no effective therapeutic treatments against osteoarthritis are known. It is therefore imperative to dissect the underlying mechanism of disease aetiology as this may enhance effective and targeted drug development.

OA has a strong genetic component. Depending on the joint affected, the heritability of OA is estimated in the range of 40-60% (7,8), with hand OA having the largest heritability, i.e. ~60% (9, 10). Therefore, in recent years, several large-scale genetic studies have been performed to identify the underlying genes and pathways leading to OA. Multiple significant associated loci for OA of the hip and knee have been identified through genome-wide association studies (GWAS) (11–18). However, thus far, only one report has described a robust association with OA of the hand (19). In this previous report, common variants in the *ALDH1A2* and rare variants in chromosome 1p31 were genome-wide significantly associated with hand OA using a discovery cohort of 837 cases and 77,325 controls.

In this study, we aimed to identify novel genes and pathways involved in the aetiology of OA of the hand by performing a large-scale GWAS. We used a semi- quantitative measure for OA of the hand in order to increase statistical power. We gathered a large sample size of 12,754 individuals for analysis, by combining data from three studies in the discovery phase and an additional three cohorts for replication. Next, we conducted functional follow-up of our top finding to investigate the underlying mechanism.

METHODS

DISCOVERY GWAS, REPLICATION AND META-ANALYSIS

For a detailed description on the GWAS methods, participating studies, quality control procedures for genotyping and imputation, see **Supplementary Text S1** and **Supplementary Table S1**.

DETAILED PHENOTYPE DESCRIPTION OF KL SUM-SCORE

We have used a semi-quantitative bilateral measure of osteoarthritis of the hand based on the radiographic Kellgren and Lawrence score (KL-score) (20). Using radiographs of both hands, the KL-score was determined for each joint in the hand. Using these KL-scores we defined the KLsum-score: the total KL-score, the sum, of the following hand joints for both hands (left and right): all Distal Interphalangeal (DIP) joints, all Proximal Interphalangeal (PIP) joints, all Metacarpophalangeal (MCP) joints, the Interphalangeal (IP) joint and the first Carpometacarpal (CMC1) joint. Which gives the sum of 15 joints on each hand, and in total 30 joints for both hands together, resulting in a minimum score of 0 and a maximum score of 120. The Leiden Studies cohort no Kellgren-Lawrence scoring was done of the MCP joints, resulting in a KLsum-score of maximum 88. Individuals lacking KL-grading for both or one hands and individuals with missing age or gender information were excluded from all analyses in all cohorts. As the KL-sum score has a skewed distribution the top finding of the meta-analysis was repeated in the discovery cohorts using a Poisson regression.

VISUALIZATION OF THE ASSOCIATED LOCI AND THE REGULATORY LANDSCAPE

For the top GWAS associated SNP, the LD region ($r^2 > 0.8$) was determined using the 1000G Phase-1 population using the HaploReg V3 tool (21). Using the ROADMAP generated reference epigenomes we determined if any of the variants in high LD were located in potential gene regulatory regions in primary osteoblasts (generated by ENCODE) and bone marrow derived chondrocytes (ROADMAP) (22,23). The 18-state chromatin reference epigenomes were downloaded from the ROADMAP epigenomes data portal (23). SNPs and regulatory annotations were visualized using the UCSC genome-browser on GRCh37/hg19 (24). For each variant it was also determined if the alternative allele would disrupt a protein binding motif, this was done using the HaploReg V3 tool (21).

RNA-SEQUENCING DATA

Post RNA isolation (Qiagen RNeasy Mini Kit, RIN > 7) of 40 knee (15 paired preserved (P) and OA lesioned (OAL), 7 P only, 3 OAL only) and 28 hip (6 paired P and OAL, 14 P only, 2 OAL only) cartilage samples (**Supplementary Table S2**), paired-end 2x100bp RNA library sequencing (Illumina TruSeq RNA-Library Prep Kit, Illumina HiSeq2000) resulted in an average of 10 million fragments per sample. Reads were aligned using GSNAP against GRCh37/hg19, in which SNPs from the Genome

of the Netherlands consortium with MAF>1% were masked to prevent alignment bias. Number of fragments per gene were used to assess quantile-adjusted conditional maximum likelihood (qCML) (edgeR, R-package). Subsequently, differential gene expression analysis was performed pairwise between P and OAL samples for which we had RNA of both (n=21). ASE was assessed using SNVMix2 (25) with default settings (min coverage=25, 10 reads per allele). The extent of allele specific expression (ASE) was defined as the fraction of risk allele among all counts at the respective location. Meta-analysis was done only across P samples or OAL when no P counterpart sample was present. P-values were calculated using canonical binominal test (metagen R-package).

TAQMAN ASSAY

Conventional TaqMan genotyping was performed on both genomic-DNA (gDNA), articular cartilage and Subchondral bone cDNA. An allele-specific custom TaqMan assay for rs1800801 (Thermo Fisher Scientific) was used to quantify the allele ratio in cDNA samples and were normalized against the gDNA ratio, which was used as an 1:1 allele ratio reference. Each sample has been measured in four (cartilage) or eight (subchondral bone) times, while calculations and statistics were performed as described previously (19,26). Cartilage samples which yielded fewer than four measurements (N=2) were discarded prior to further analyses. All subchondral bone samples were assessed by eight technical replicates.

RESULTS

GWAS OF KLSUM-SCORE

We conducted a genome-wide association study (GWAS) of a semi-quantitative measure of hand OA, a bilateral summed score of Kellgren- and Lawrence scores (20), that grades radiographic OA severity, across all hand joints (Klsum-score, range of 0 to 120). The discovery set consisted of three Rotterdam Study cohorts (RSI, RSII and, RSIII) and included 8,743 participants with Klsum-scores. Replication was done in another 4,011 individuals from three different cohorts; Leiden studies(LS), Framingham heart study(FHS), and Twins-UK (TUK). General characteristics of the discovery cohorts and replication cohorts can be found in **Supplementary Table S3** and in **Supplementary Text S1**.

The discovery analysis yielded two novel independent genome-wide significant loci ($P \leq 5 * 10^{-8}$) on chromosome 12, an intergenic region between *MGP* and *ERP27* and an intronic region in *CCDC91*. We also identified seven other novel loci with suggestive significance ($P < 5 * 10^{-6}$) (**Figure 1**). In total, nine loci were selected for replication in 4,011 individuals from three different cohorts (LS, FHS, and TUK). Using a Bonferroni corrected P-value $< 5.56 * 10^{-3}$, we significantly replicated one of nine loci,

rs4764133 ($\text{Beta}_{\text{meta}}=0.83$, $\text{SE}_{\text{meta}}=0.10$, $\text{P-value}_{\text{replication}}=3.4*10^{-7}$, $\text{P-value}_{\text{meta}}=1.8*10^{-15}$) with the same direction of effect as identified in the discovery analysis (**Table 1**, and **Supplementary Figure S1**). This locus maintained genome-wide significance and another locus near *ENPP3* reached near genome-wide significance (chr6:132063842:D, $\text{Beta}_{\text{meta}}=0.58$, $\text{SE}_{\text{meta}}=0.11$, $\text{P-value}_{\text{meta}}=3.8*10^{-7}$) in the combined discovery and replication joint meta-analysis (**Table 1**). Since the KLsum-score has a skewed distribution the top hit was also re-analysed in the discovery set using a Poisson regression (rs4764133, $\text{Beta}_{\text{poisson}}=0.12$, $\text{SE}_{\text{poisson}}=0.02$, $\text{P-value}_{\text{poisson}}=1.98*10^{-11}$).

Our top replicated and genome-wide significant finding, rs4764133 [T] ($\text{P}_{\text{meta}}=1.80*10^{-15}$, $\text{Beta}=0.83$, $\text{MAF}=0.39$) is located in a non-coding intergenic region between *MGP* (Matrix Gla-protein) and *ERP27* (Endoplasmic Reticulum Protein 27). However, variants in high linkage disequilibrium (LD) with rs4764133 ($r^2 \geq 0.8$) span a ~80Kb region encompassing multiple genes, including *MGP* and an open-reading frame *C12orf60* (**Figure 2A**). Moreover, several of these variants are located in an mRNA transcript, including a nonsynonymous variant in *MGP*, and variants in 3' and 5'UTR of *MGP* and *C12orf60* (**Table 2, Figure 2B**). The nonsynonymous variant in *MGP*, rs4236, is predicted to be non-damaging (STIFT=1, tolerated; PolyPhen=0, benign) causing a threonine to alanine amino acid substitution. Two variants are located in predicted active promoter region of *MGP* (rs1800801) and *C12orf60* (rs9668569) in chondrogenic cells and primary osteoblasts (**Table 2**).

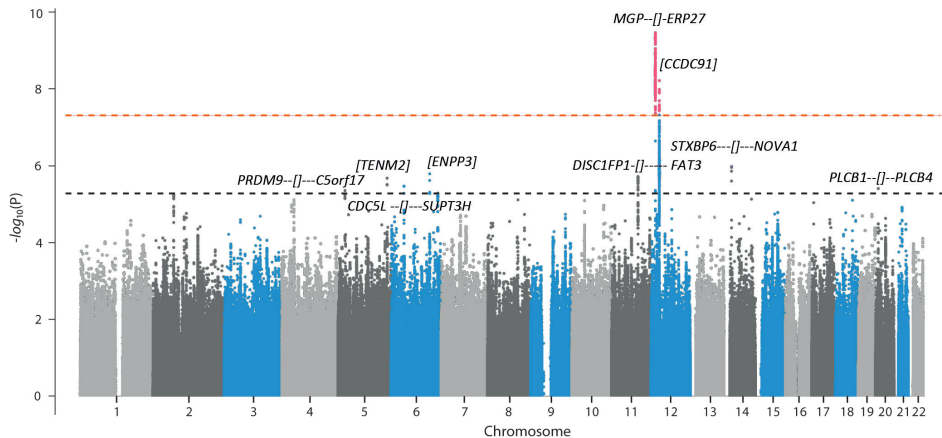


Figure 1. GWAS results for association with the KLsum score in the discovery phase. Manhattan plot for association with the KLsum-score, adjusted for age and sex, in the discovery cohorts of RSI, RSII and RSIII. The $-\log_{10}$ P-values, for each of the ~11 million SNPs analyzed (remaining after EASYQC quality control) as part of the genome wide association with the KLsum-score, plotted against their position per chromosome. The red dotted horizontal line corresponds to the genome-wide significant threshold ($P = 5 \times 10^{-8}$). The dotted grey line corresponds to the selection for replication threshold ($P = 5 \times 10^{-6}$). SNP location represented by [], if the SNP is localized intergenic the dashes denotes the distance, ≤ 10 kb, ≤ 100 kb, ≤ 1000 kb, ≤ 1 Mkb, ≥ 1 Mkb.

Table 1. Results GWAS quantitative bilateral phenotype of osteoarthritis of the hand (Klsum-score), discovery, replication and meta-analysis

SNP	Chr	Position (hg19)	Effect Allele	Other Allele	EAF†	Discovery*			Replication**			Combined			Genomic location††
						Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	
rs1494593	5	23574856	T	C	0.88	-0.83	0.18	4.46E-06	-0.15	0.32	6.31E-01	-0.67	0.16	2.28E-05	PRDM9-[]---C5orf17
rs114370021	5	167398535	A	G	0.27	-0.84	0.18	2.12E-06	0.48	0.32	1.28E-01	-0.53	0.16	6.96E-04	[TENM2]
rs7770034	6	44447004	A	G	0.48	-0.54	0.12	3.42E-06	-0.36	0.21	8.71E-02	-0.50	0.10	1.01E-06	CDC5L-[]---SUPT3H
6:132063842:D‡	6	132063842	D	I	0.27	0.64	0.13	1.28E-06	0.41	0.23	7.82E-02	0.58	0.11	3.79E-07	[ENPP3]
11:90657297:D	11	90657297	D	I	0.11	0.93	0.19	1.35E-06	0.33	0.33	3.11E-01	0.78	0.17	2.91E-06	DISC1FP1-[]---FAT3
rs4764133	12	15064363	T	C	0.39	0.75	0.12	3.45E-10	1.11	0.22	3.34E-07	0.83	0.10	1.80E-15	MGP-[]-ERP27
rs7139060	12	28693144	A	G	0.67	-0.73	0.12	6.12E-09	0.11	0.22	6.16E-01	-0.52	0.11	1.47E-06	[CCDC91]
rs1950427	14	25955502	T	C	0.12	0.86	0.18	1.09E-06	-0.28	0.30	3.56E-01	0.57	0.15	1.80E-04	STXBPF6---[]---NOVA1
rs6108226	20	8960884	T	C	0.77	0.70	0.15	3.94E-06	0.20	0.27	4.43E-01	0.58	0.13	1.14E-05	PLCB1-[]--PLCB4

* Discovery: RS-I, RS-II, RS-III, $n = 8,743$ ** Replication : GARP LSS, TwinsUK & FHS, $n = 4,011$

† EAF: Effect Allele Frequency

††SNP location represented by [], if the SNP is localized intergenic the dashes denotes the distance, ≤ 10 kb, ≤ 100 kb, ≤ 1000 kb, ≤ 1 Mkb, ≥ 1 Mkb#For Twins UK a proxy SNP was used: rs3850251, $r^2 = 1$ D' = 1 (as calculated in the RSI, RSI and RSIII cohorts)

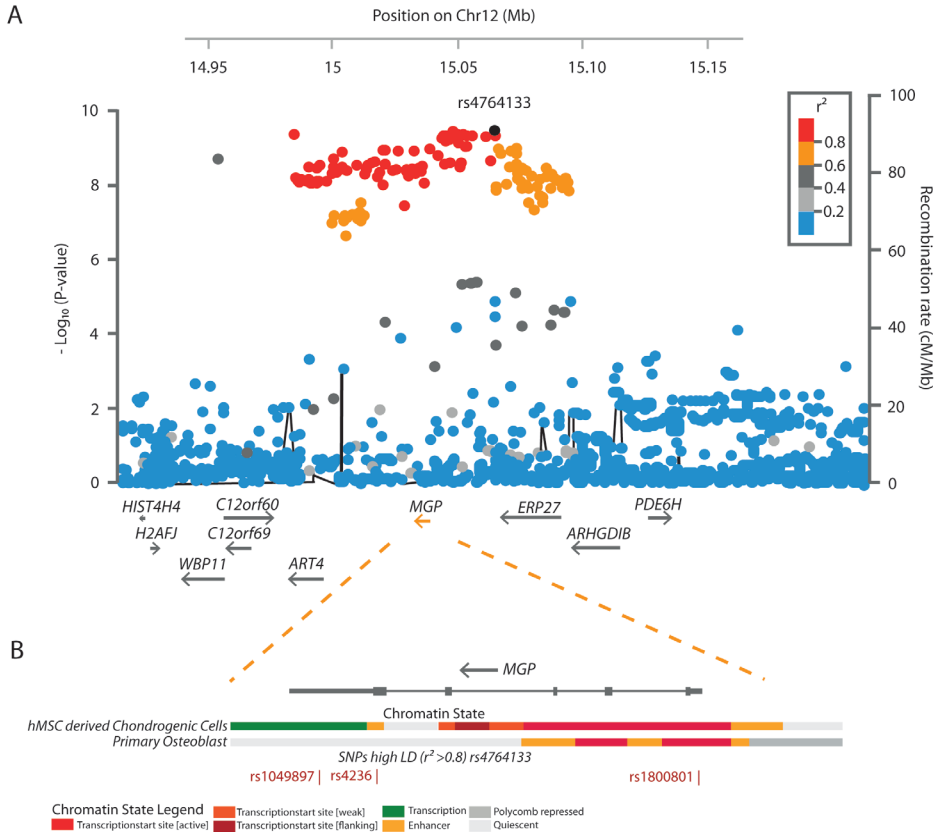


Figure 2. Locus zoom plot for rs4764133. locus zoom plot for rs4764133, 150 kb upstream and downstream of rs4764133 has been taken as plotted region (**A**). Zoom in on *MGP* and three SNPs in high LD with top SNP that are located in the *MGP* mRNA transcript (**B**). Also represented is ROADMAP chromatin 18-state data of two tissue types: human Mesenchymal Stem Cell (hMSC) derived cultured chondrogenic cells and primary osteoblasts. In both these cell types the chromatin contains active marks surrounding the *MGP* promoter.

Table 2. rs4764133 LD block ($r^2 > 0.8$) annotation of potential functional elements in osteoblasts and chondrogenic cells, X marks no potential functional annotation i.e. enhancer region, promoter region or altered protein binding motifs.

SNP	P-value Discovery	r ²	Annotation*	Regulatory Chromatin Marks**		Altered Protein Binding Motifs (Haploreg V3)
				Chondrogenic cells	Osteoblasts	
rs1049897	3.48E-09	0.88	<i>MGP</i> 3'-UTR	Transcription	X	X
rs4236	4.16E-09	0.86	<i>MGP</i> non-synonymous	Enhancer region	X	HNF4, PLAG1
rs1800801	1.12E-09	0.95	<i>MGP</i> 5'UTR	Promoter region	Promoter region	Zfp410
rs7310951	4.04E-09	0.86	<i>C12orf60</i>	Enhancer region	X	DMRT7, Gfi1, Pax-5
rs12320004	4.04E-09	0.86	<i>C12orf60</i>	Enhancer region	X	BHLHE40, P300, HEN1, LBP-1, RAD21, TATA, Zfx
rs10772814	3.76E-09	0.88	<i>C12orf60</i>	Enhancer region	X	HNF4
rs10492151	1.21E-09	0.95	<i>C12orf60</i>	Enhancer region	X	AIRE, Hoxa13
rs725445	3.58E-08	0.82	<i>C12orf60</i>	Enhancer region	X	Hand1
rs725444	3.92E-09	0.87	<i>C12orf60</i>	Enhancer region	X	Foxf1, Foxi1, Foxo, Foxq1, Mef2
rs4764131	6.31E-10	0.97	<i>C12orf60</i>	Enhancer region	Enhancer region	Myc
rs9668569	5.91E-10	0.97	<i>C12orf60</i>	Promoter region	Promoter region	X
rs2430687	2.44E-09	0.89	<i>C12orf60</i>	Enhancer region	Enhancer region	BHLHE40
rs12311463	6.91E-10	0.97	<i>C12orf60</i>	Enhancer region	Enhancer region	Pou1f1, Pou2f2, TATA
rs67482087	4.61E-10	0.95	<i>C12orf60</i>	Enhancer region	Enhancer region	Foxp1, Irx, Pou1f1, Pou2f2, Pou3f3, TATA
rs67436073	6.76E-10	0.97	<i>C12orf60</i>	Enhancer region	Enhancer region	Foxj2, Foxk1, Foxo, GATA, Mef2, Pou2f2, Pou3f2, Pou6f1, TATA, Zfp
rs11276	8.05E-09	0.96	<i>C12orf60</i> non-synonymous	X	X	SPIB, NF-AT
rs3088189	9.46E-09	0.96	<i>C12orf60</i> synonymous	X	X	SPIB
rs1861698	3.56E-09	0.96	<i>C12orf60</i> synonymous	X	X	Bbx, Pou1f1, TATA

*Gene annotation based on the hg19 release of the UCSC Genome Browser

** Regulatory chromatin marks taken from the ROADMAP Epigenomes project chromatin state learning core 18-state model

Next, we investigated the association of rs4764133 with bilateral severe hand OA and bilateral finger OA using the discovery set (RS-I, RS-II and RS-III). We found a strong association with finger OA (P -value= 3.09×10^{-8} , OR=1.25) and nominal significant association with severe hand OA (p -value= 2.80×10^{-2} , OR=1.36), which has a low frequency in the population (**Supplementary Table S4**), we also see a nominal significant association with cartilage thickness in the hip joint (minimal joint space width (mJSW)). To see if rs4764133 also confers risk for other forms of osteoarthritis, i.e. osteoarthritis of the hip and knee, we used the GWAS summary data of the TreatOA consortium (27) and the recently published mJSW meta-analysis (18). No association was found between rs4764133 and hip or knee OA (**Supplementary Table S4**). However, we did find a nominal significant association for rs1049897 ($r^2 = 0.98$ with rs4764133)(P -value= 1.28×10^{-2} , Beta=-0.398).

GENE EXPRESSION ANALYSES

In order to identify potential causal genes located in the LD block surrounding rs4764133, we assessed gene expression of *MGP*, *ERP27*, *ART4*, *SMOC3* (*C12orf69*) and *C12orf60* in articular cartilage, the primary OA affected tissue. RNA sequencing was obtained on articular cartilage from primary OA patients who had total joint replacement surgeries of either the knee ($n=25$) or hip ($n=22$) joint. Expression levels of *ERP27*, *C12orf60*, *ART4* and *SMOC3* were substantially lower than *MGP* expression levels in articular cartilage (**Supplementary Figure S2A**). Nonetheless, neither *MGP*, *ERP27*, *ART4* nor *SMOC3* and *C12orf60* showed significant difference in gene expression between paired preserved(P) and OA lesioned(OAL) articular cartilage. However, while these genes are not differentially expressed in OA affected cartilage, it is possible that the identified GWAS SNPs affect gene transcription. When we analysed the relationship between the top SNP and expression analysis in a classical eQTL (expression Quantitative Trait Loci) analysis, we did not detect significant correlations between rs1049897, rs4236 or rs1800801 and absolute *MGP*, *ERP27*, *ART4*, *SMOC3* or *C12orf60* expression levels (**Supplementary Figure S2B**). However, we did observe several variants in high LD located in the mRNA transcript of *MGP* and *C12orf60*, allowing us to assess allele specific expression (ASE) for these genes. We were unable to study ASE for *ART4*, *SMOC3* and *ERP27*, since no SNP in high LD with rs4764133 is present in the coding region. In ASE the influence of exonic alleles on gene expression *in-cis* is measured within heterozygote subjects, circumventing strong effects from environmental or trans-acting influences. This property results in ASE analysis to be a more statistically powerful approach, when compared to classical eQTL analysis (28). Subsequently, we found that the OA risk alleles for three coding variants in high LD with the lead variant, rs4236 (**Supplementary Figure 3SA**, 39.6% C allele, $P < 5 \times 10^{-16}$), rs1049897 (**Supplementary Figure S3B**, 44.4% A allele, $P < 5 \times 10^{-10}$), and rs1800801 (**Figure 3A**, 40.7% T allele, $P < 5 \times 10^{-16}$), were significantly correlated with lower expression of *MGP*, marking imbalanced expression among heterozygotes, independent of the disease status of the articular cartilage. No allele specific expression was observed between SNPs

rs11276, rs3088189 and rs1861698 (residing in *C12orf60* and in high LD with the lead SNP, $r^2 > 0.8$, **Table 2**). Technical and biological replication was performed using a custom allele specific TaqMan assay for rs1800801 in eight additional heterozygous individuals for which we isolated RNA from P cartilage (n=2), OAL (n=2) or both (n=4) from primary knee OA patients and confirmed the observed imbalance in preserved articular cartilage (**Figure 3B**, relative allelic difference=0.92, $P < 1 \times 10^{-6}$), as well as in 8 knee subchondral bone samples (**Figure 3C**, relative allelic difference=0.78, $P < 1 \times 10^{-4}$).

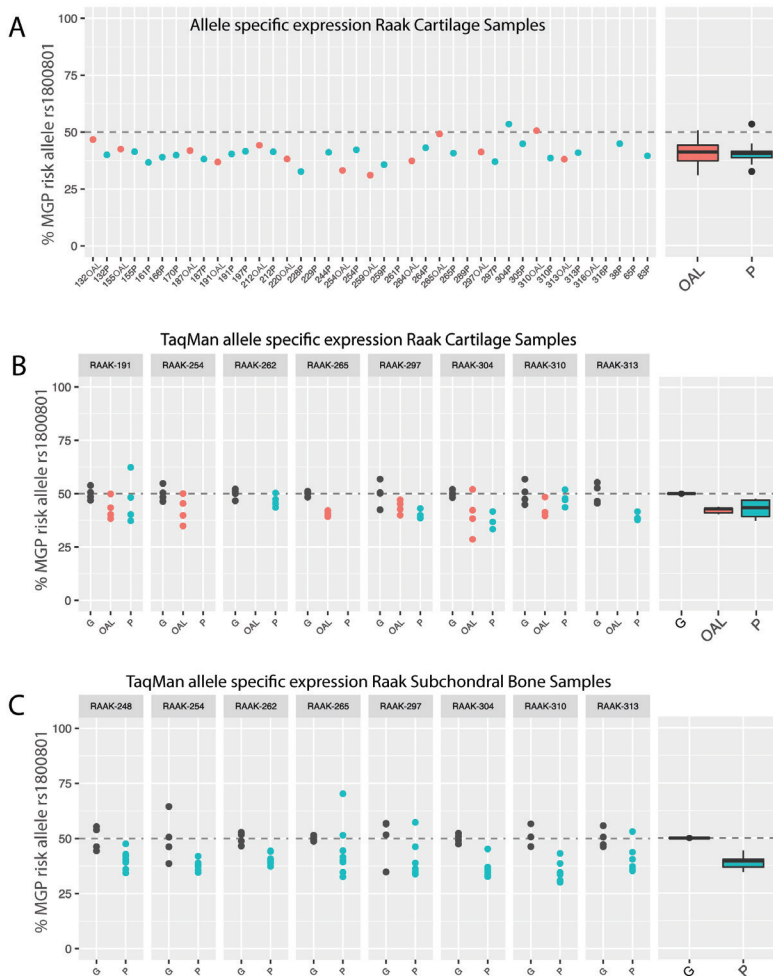


Figure 3. Allelic imbalanced expression of *MGP* marked by the alleles among heterozygotes of rs1800801 (**A**), in the assessed cartilage RNA sequencing dataset. Validation of selected rs1800801 using a custom TaqMan assay confirmed the imbalance (**B**). Allelic imbalance was also assessed in with a custom TagMan assay in subchondral bone samples (**C**). Preserved (P) and OA lesioned (OAL) samples are shown respectively in blue and red, and genomic DNA (TaqMan control) in black (G). For ASE results for rs4236 and rs1049879, see **Supplementary Figure S3** and for information on the samples see **Supplementary Table S2**.

DISCUSSION

Here, we show for the first time, that there is a robust genome-wide significant association between rs4764133, located near *MGP*, and hand OA. Furthermore, we performed functional validation showing that *MGP* coding variants in LD with rs4764133 are associated with allele-specific expression of *MGP* which may increase risk of hand OA by lowering inhibition of articular cartilage calcification, since *MGP* is an essential inhibitor of cartilage calcification (29,30). These findings suggest that *MGP* could be considered a prioritized drug target for hand OA, since genetically supported drug targets double the success rate of therapeutics in clinical development (31).

MGP is an essential inhibitor of cartilage calcification, and genetic deficiencies of *MGP* in humans and mice have been linked to abnormal mineralization of soft tissues, including cartilaginous tissue (29,32). Furthermore *MGP* has been previously implicated in relation to OA. A small candidate study reported marginally significant association between hand OA and genetic variants in *MGP* (rs1800802 and rs4236) (33). This is consistent with our findings that the minor allele for rs4764133 and related coding variants in high LD ($r^2 > 0.8$), rs1800802 and rs4236, increase the risk of hand OA and that we found high expression of *MGP* in both preserved and OA lesioned articular cartilage. In contrast, another study showed that an *MGP* protein complex is excreted by healthy articular chondrocytes, but not by OA affected chondrocytes (34), although we only assayed *MGP* expression and not *MGP* protein complex excretion.

Although the loci with allele specific expression (ASE) are known to be enriched for eQTLs (35), we were unable to detect an association between the *MGP*-genotype and *MGP* RNA-expression levels in cartilage. This could have been due to our modest sample size (knee joint, n=25 and or hip joint, n=22) in combination with large heterogeneity of the tissue. Notably, the available cartilage samples originated from different joint sites (knee, hip) and different disease stage (preserved versus affected), and had large age range of the individuals. Also, it is known that ASE is a more powerful technique than classical eQTL analysis to identify functional SNPs influencing expression of genes (28). While the extent of imbalance could be considered relatively modest, an increasing number of OA associated SNP alleles appear to mark ASE by comparable amount (19,36–38). From a more biological perspective, one could consider a prolonged, albeit slight, deviation from homeostasis due to modest ASE of cartilage relevant genes to be of substantial influence over time. This latter hypothesis could contain the molecular basis for increased risk towards developing OA among the ageing population. Additionally, we observed that the rs1800801 alleles also affected expression of *MGP* in subchondral bone samples. This could imply that, in parallel to an effect in cartilage, the presumed disturbed cartilage homeostasis is further affected by the underlying bone. Further enabling the view that OA is a pathology of the entire joint.

Our findings may give an explanation for the known vitamin K association with OA: MGP mediated calcification inhibition is dependent on γ -carboxylation by vitamin K (39). It has been shown that low vitamin K intake is correlated with OA (40). Thus vitamin K intake may be a potential therapeutic treatment in OA. Recently, a first randomized control trial testing the effects of vitamin K on OA was published, which reported no overall effect of vitamin K on hand OA (41). Despite the low power of the trial, there was a significant beneficial effect on joint space narrowing (cartilage degradation) among those individuals that were vitamin K deficient at the start of the trial (41). Thus, an adequately powered study of vitamin K may be justified based on the found *MGP* association. Furthermore, genetic predisposition for hand OA, was not taken into account in the trial. Perhaps, genetic predisposition for hand OA (*MGP*-risk variants) in combination with insufficient vitamin K intake might potentiate cartilage calcification and subsequent risk for developing hand OA. Therefore, future OA trials, therapeutic and preventive treatments might benefit from taking a personalized medicine approach since genetically supported drug targets double the success rate of therapeutics in clinical development (31).

Styrkarsdottir *et al.* (2014) reported on common genetic variants that associate with severe hand OA, among the replication cohorts were the Leiden and Rotterdam cohorts (19). Although we observe suggestive signals at the reported locus (*ALDH1A2* gene, 1p31) the respective variants did not meet the genome-wide significance threshold in our analyses (**Supplementary Table S5**). This difference is likely caused by the markedly different phenotypes that were used for either analyses. Where Styrkarsdottir *et al.* studied a dichotomous severe hand OA phenotype, our phenotype was semi-quantitatively phenotype.

To conclude, we here present coding variants in *MGP*, that are associated with radiographic hand OA, and the hand OA risk allele marks lower expression of *MGP* in articular cartilage. Our findings suggest that *MGP* might play an important role in hand OA pathogenesis through pathways related to articular cartilage calcification and vitamin K. Better understanding of *MGP* gene and protein regulation and its relation to vitamin K intake and OA, may reveal novel therapeutic drug targets for hand OA.

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AUTHOR CONTRIBUTIONS

W.H. and C.G.B. contributed equally to this work. D.H., M.S.Y., Y.F.M.R. and S.M. performed replication analysis for this work, L.B. provided analysis help. L.S.C. and F.R. provided data. M.K. provided phenotypic contribution to the GARP study. M.P. provided data and analyses. T.D.S. contributed data for replication. A.H. contributed data of the RS cohorts. J.D., M.B. and P.E.S contributed to genotyping data and analyses of LLS cohort. R.G.H.H.N. provided contribution to the RAAK study. A.G.U. contributed genotype data of RS cohorts, D.T.F. and A.V. contributed replication data for this work. I.M., and J.B.J.M. jointly supervised this work.

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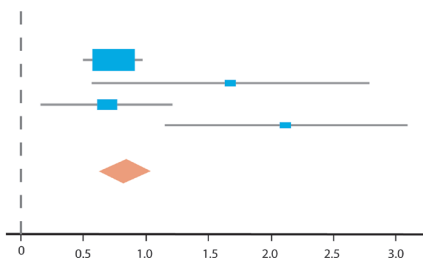
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Forrest Plot rs4764133

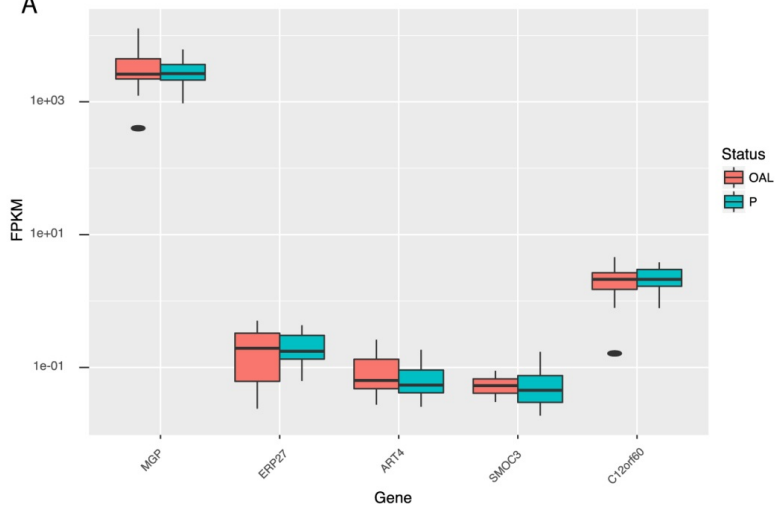
Study name	<i>n</i>	Beta (interval)
Rotterdam Study (RS1-3) (the Netherlands)	8743	0.75 (0.63-0.87)
Leiden Hand OA Studies (the Netherlands)	836	1.68(1.11 -2.24)
Twins UK (United Kingdom)	2000	0.69(0.42-0.96)
Framingham Heart Study (United States of America)	1175	2.12(1.62-2.61)

Total 12754 0.83 (0.73-0.93)
 Heterogeneity: $\chi^2=63.27$, $df=3$ ($P=0.02$), $I^2=69.07$

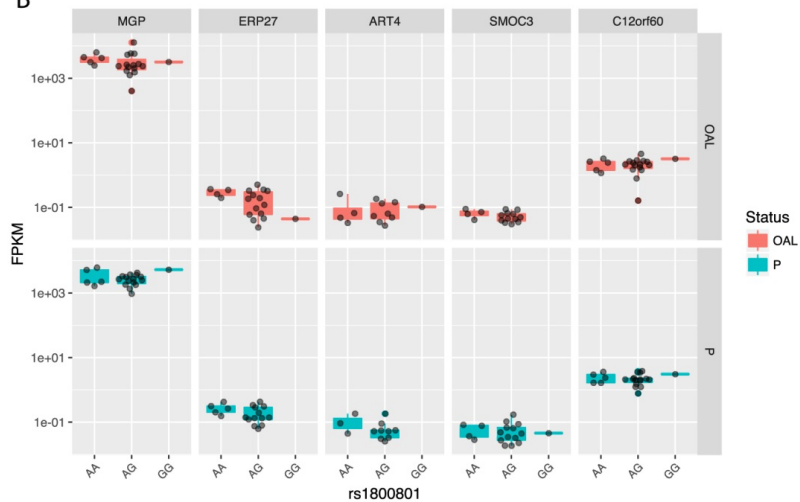


Supplementary Figure S1.

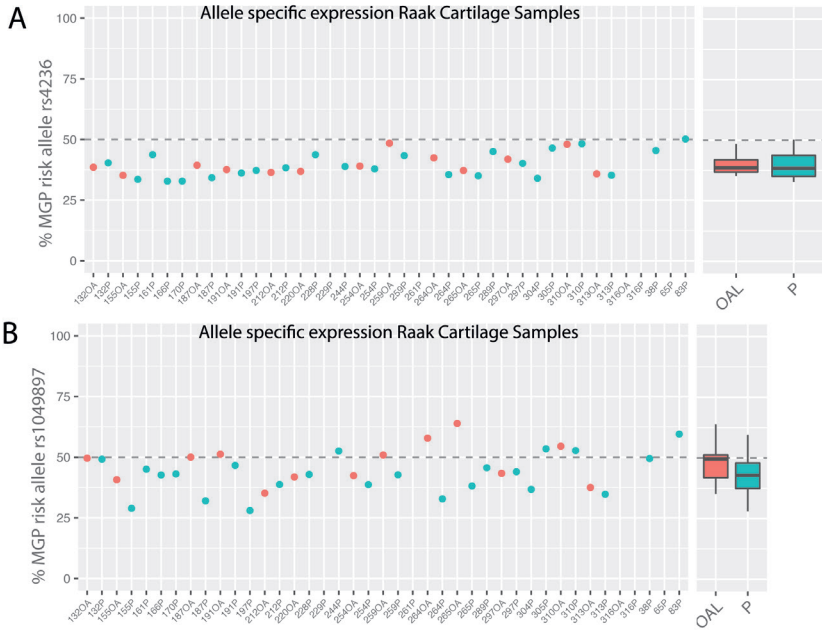
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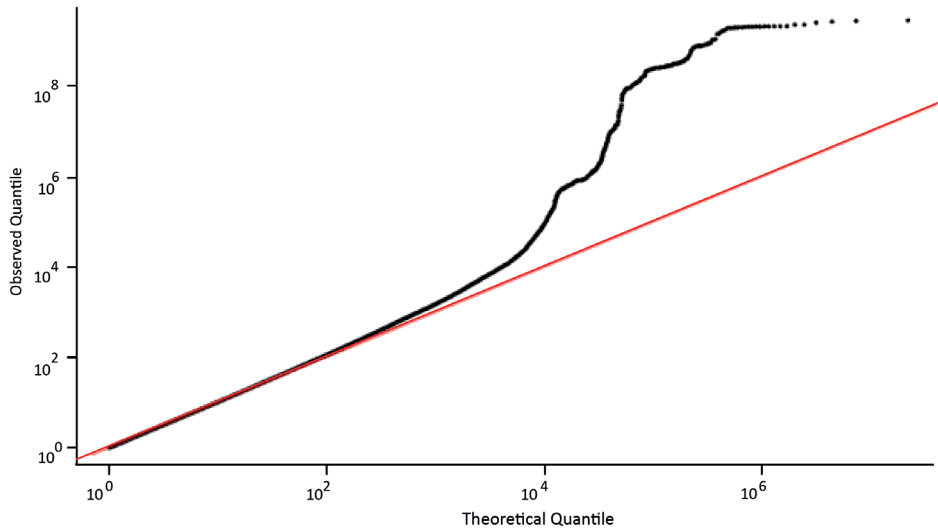
B



Supplementary Figure S2.



Supplementary Figure S3.



Supplementary Figure S4.

