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A genome-wide association study suggests that a locus within the ataxin 2 binding protein 1 gene is associated with hand osteoarthritis: the Treat-OA consortium

Running title: A2BP1 gene and hand OA

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Abstract

To identify hand OA susceptibility gene, we utilized a two-stage approach genome-wide association study (GWAS) using two discovery samples (the TwinsUK cohort and the Rotterdam discovery subset; a total of 1804 individuals) and four replication samples (the Chingford study, the Chuvasha Skeletal Aging study, the Rotterdam replication subset, and the GARP Study; a total of 3266 individuals). 5 SNPs suggested the likelihood of association with hand OA in the discovery stage and one of them – rs716508, was successfully confirmed in the replication stage (meta-analysis $p=1.81 \times 10^{-5}$). The C allele conferred a reduced risk of 33-41% using a case-control definition. The SNP is located in intron 1 of the A2BP1 gene and we also found that the same allele of the SNP significantly reduced bone density at both the hip and spine ($p < 0.01$), suggesting the potential mechanism of the gene in hand OA might be via effects on subchondral bone. Our findings provide a potential new insight into genetic mechanisms in the development of hand OA.

Osteoarthritis (OA) is the most common form of arthritis and a leading cause of musculoskeletal disability in middle aged and older people ¹. The hand is one of the most frequent OA-affected joints. Hand OA is more common in women and is significantly associated with functional impairment and reduced independence². While it has been associated with age and environmental factors such as occupation, hand OA has a significant genetic component with a heritability estimate of 65% from a twin study ³. There have been several genome-wide linkage scans reporting suggestive linkage regions on several chromosomes, but only two genes - AGC1 ⁴⁻⁶ and HFE ^{7;8}, have been reported to be associated with hand OA in at least two independent samples. However, inconsistent case definitions and sample size make the interpretation of the results inconclusive.

Genome-wide association study (GWAS) is a powerful tool for unlocking the genetic basis of complex diseases such as hand OA. The approach has been used successfully in several common diseases⁹. Notable advantages include its comprehensiveness and the potential for finding susceptibility genes with previously unknown loci and relationship to the disease. In the current study, we carried out this GWAS for hand OA.

As a discovery sample, we used 2277 individuals of European ancestry (1073 singletons and 602 dizygotic twins (DZs)) from the TwinsUK registry genotyped using with the Illumina Hap317K chip (Illumina, San Diego, USA). We applied a strict quality control at both individual and SNP levels. We excluded 51 individuals due to non-European ancestry and 3366 SNPs due to the call rate < 95% , MAF < 1%, or $P_{HWE} < 1 \times 10^{-4}$ (details are provided in the supplementary methods).

After the quality control, 305,811 autosomal SNPs available on 2226 individuals (1046 singletons and 590 DZs) were available. Of these, 799 females had X-rays for both hands. The distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), and first carpometacarpal (CMC) joints of the thumb were assessed for radiographic OA according to Kellgren/Lawrence (KL) score using a standard atlas¹⁰ (details are provided in supplementary methods & supplementary table 1). We summed each joint's KL score and used the total hand KL score as the outcome measure of hand OA. We adjusted the total hand KL score for age using regression model and used the normalized residuals as a quantitative measurement of hand OA and performed the GWAS analysis using the score test implemented in Merlin¹¹ which took account of relatedness (see details in supplementary methods). None of the tested SNPs achieved P values small enough to be considered as genome-wide significant with a conservative Bonferroni correction for multiple testing. We therefore selected the top 100 SNPs (supplemental table) with $P \leq 3.6 \times 10^{-4}$ and sought confirmation in an available cohort with both genotype and hand OA data – a subset of the Rotterdam Study. The quantile-quantile (QQ plot) for p values (Figure 1) indicated that SNPs with $P \leq 3.6 \times 10^{-4}$ are likely to be real genetic associations because the observed p values deviate from the expected.

The Rotterdam Study is a prospective population cohort of Dutch men and women aged 50 or above. There were initially 1005 women with hand OA data available from the cohort genotyped using Illumina Hap550K array (details are provided in the supplementary methods). The DIP, PIP, MCP, and the first CMC joints of both hands were assessed using KL score system and the total KL score after adjustment for age was used in the discovery cohort analysis. Five of the SNPs we

selected (rs1334995, rs2443547, rs1958654, rs938076, and rs716508) on 5 chromosomes were confirmed with $p \leq 0.05$ in the Rotterdam sample (Table 1).

However, the effect direction of these 5 SNPs was not the same between the TwinsUK cohort and the Rotterdam discovery cohort for 4 out of these 5 SNPs, to rule out the spurious associations, we genotyped all these 5 SNPs in another independent sample – the Chingford study, which is a well-described 20 year prospective population-based longitudinal study of osteoarthritis and osteoporosis, comprising 1003 women aged 43 or above at entry derived from the age/sex register of a large general practice in Chingford, North London, who are seen annually and described in detail previously^{12; 13}. The presence of hand OA was assessed in the same manner as in our TwinsUK cohort and was performed by the same readers (DH). The analysis included 637 women with both the hand OA and genotype data available. Among the 5 SNPS, rs716508 was significantly associated with hand OA ($p = 0.004$) (table 2). The significance persisted even after Bonferroni correction for multiple testing in this replication sample. The other four SNPs were not significant with $p = 0.20 - 0.96$.

To further confirm this significant association, we replicated in three other samples – the Chuvasha Skeletal Aging study, the remainder of the Rotterdam cohort, and the GARP Study (more details about these three cohorts in supplement). The significance was strongest in the Chuvasha study but borderline in the Rotterdam remaining sample (Table 2). Although the results in the GARP study were not significant, a similar effect was obtained (Table 2). **The non-significant results in the GARP study might be due to the association being gender-specific, as**

the significant results are found in all the female cohorts (the TwinsUK, the Rotterdam discovery sample, the Chingford study, and the Chuvasha study). Indeed, when we restricted the analysis in GARP only to females, the p value tended to be smaller ($p = 0.10$) and the effect size became larger ($\beta = -0.14$). However, the association is significant in males ($p=0.039$) but not in females ($p=0.485$) in the Rotterdam replication sample. An independent study is needed to clarify the sex-specific effect. Another possible explanation is simply small sample size.

We meta-analysed the summary results for the SNP rs716508 using both discovery cohorts and replication cohorts with a fixed effect model and inverse-variance weighted averages of β coefficients. The pooled effect of allele C which is the minor allele was associated with -0.09 grade of the total KL score after adjustment for age (95% CI $-0.14, -0.05$) with a p value of 4.75×10^{-5} . There was a significant between-study heterogeneity ($p < 0.001$). This is mostly due to the Rotterdam subset used in the discovery stage, for which the effect direction of the C allele was opposite to the other 5 samples. The reason for this discrepancy is unclear, possible explanations include chance or random effects. When we excluded the discovery samples, the pooled effect estimate was -0.12 (95% CI $-0.18, -0.06$) with $p = 1.81 \times 10^{-5}$ (Table 2) (supplemental figure 1) and there was no between-study heterogeneity ($p=0.29$).

In addition, we examined the association between rs716508 and hand OA in a case-control fashion using the TwinsUK and the Chingford cohorts who had X-rays read by the same observer. When we categorized the people as hand OA cases if they had at least two hand joints affected, defined as $KL \geq 2$, the C allele was associated with a 33% reduction of risk in the

development of hand OA ($p=2.0\times 10^{-4}$). The protective effect is increased to 41% if the cases are defined more severely as at least three joints affected ($p=1.0\times 10^{-5}$). Similar results were obtained if excluding the discovery sample.

The SNP rs716508 is located in the intron 1 of the ataxin 2-binding protein 1 gene (A2BP1) (Supplemental figure 2). A2BP1 has an RNP motif¹⁴ that is highly conserved among RNA-binding proteins. This protein binds to the C-terminus of ataxin-2 and may contribute to the restricted pathology of spinocerebellar ataxia type 2 (SCA2). Ataxin-2 is the gene product of the SCA2 gene which causes familial neurodegenerative diseases. Ataxin-2 binding protein 1 and ataxin-2 are both localized in the trans-Golgi network. Four alternatively spliced transcript variants have been found for this gene. A2BP1 gene has been reported to be associated with autism in a subset of patients¹⁵ and smoking cessation¹⁶. However, there are no reports of the association between A2BP1 gene and OA so far.

A2BP1 gene has been reported to be a novel transcriptional regulator that mediates the neuron-specific splicing pattern of the calcitonin/calcitonin gene-related peptide (CGRP) pre-mRNA¹⁷. Immunohistochemical phenotypic characterization of skeletal nerve fibers has demonstrated the expression of a restricted number of neuropeptides including CGRP and osteoblasts and osteoclasts express functional receptors for CGRP¹⁸, suggesting potential pathways for the association between A2BP1 and hand OA. To support this hypothesis, we examined the association between the SNP rs716508 and BMD in the TwinsUK cohort of 2094 women and found that the C allele which is the protective allele for hand OA was associated with -0.07 to -

0.08 g/cm² decreases in BMD at lumbar spine and femoral neck (p = 0.01 and 0.003 for lumbar spine and femoral neck, respectively). The effects became even larger after adjustment for weight.

In addition, the A2BP1 gene is abundantly expressed in skeletal muscle and hand grip strength has also been reported to be associated with hand OA¹⁹. It could also be possible that the association between the SNP and hand OA is via muscle strength. However, the SNP was not associated with hand grip strength (p=0.20) nor the lean mass measured by DXA (p=0.515) in the TwinsUK cohort.

In summary, we have identified a novel SNP within a gene – A2BP1 on chromosome 16p13.3, which is associated with hand OA in multiple independent Caucasian samples, arguing that the findings are very unlikely to be false positive. We speculate that the potential mechanism for the association is via subchondral bone. The hypothesis is supported by the significant association between the SNP and the BMD at hip and lumbar spine. It is known that high BMD is associated with the development of OA. The C allele of the SNP is associated with reduced BMD at both hip and spine and has a protective role in hand OA.

However, we did not find any association between the SNP and hip/knee OA in the Chingford cohort, the GARP study, and the Rotterdam cohort in which we had knee and hip OA data available on the same subjects (data not shown). Although due to low power we cannot exclude an effect, this suggests a site specific gene for hand OA, which may be true given that previous

studies found that HFE gene^{7 8} was associated with hand OA but not hip or knee OA²⁰ and modelling studies confirm little pleiotropy between hand and large joint sites²¹.

Given that the SNP rs716508 is located in the intron of the A2BP1 gene, further investigations are justified to clarify the role and potential mechanism of the gene in hand OA and bone.

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Table 1. Association of the SNPs and hand OA in the discovery samples

	chr	position	Minor allele	The TwinsUK cohort			The Rotterdam discovery subset		
				MAF	β (se)	P	MAF	β (se)	P
rs1334995	1	70839082	A	0.18	-0.27(0.07)	2.3×10^{-4}	0.19	-0.12(0.06)	0.05
rs2443547	5	18209429	G	0.46	0.20(0.06)	3.6×10^{-4}	0.49	-0.11(0.46)	0.02
rs1958654	14	5329267	T	0.14	0.30(0.08)	2.4×10^{-4}	0.13	-0.13(0.07)	0.05
rs938076	15	23784012	A	0.46	0.22(0.05)	3.7×10^{-5}	0.48	-0.01(0.04)	0.01
rs716508	16	6276913	C	0.31	-0.24(0.06)	6.4×10^{-5}	0.33	0.10(0.05)	0.04

*MAF stands for minor allele frequency; β (se) stands for regression coefficient (standard error) and are expressed as changes in total KL score per copy of the risk allele after adjustment for age; p values were not Bonferroni corrected but adjusted for the relatedness in the TwinsUK cohort.

Table 2. Association of the SNP rs716508 and hand OA in replication samples

	rs716508		
	MAF	β (SE)	P value
The Chingford study	0.31	-0.18(0.06)	0.004
The Chuvasha Skeletal Aging study	0.29	-0.25(0.10)	0.009
The GARP study	0.33	-0.10(0.08)	0.20
The Rotterdam study replication subset	0.34	-0.08(0.04)	0.07
<i>Meta-analysis</i>	-	-0.12(0.03)	1.81×10^{-5}

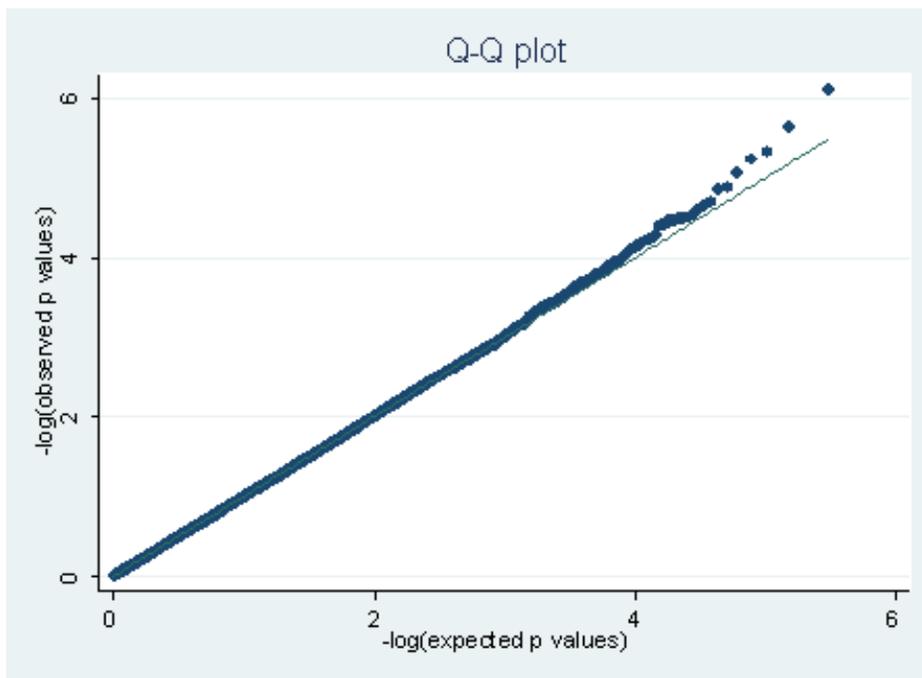


Figure 1. Q-Q plot for the GWAS analysis in the TwinsUK cohort.