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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. 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.

Key words: hand osteoarthritis; genetics; genome-wide association study

Osteoarthritis (OA) is the most common form of arthritis and a leading cause of musculoskeletal disability in middle aged and older people¹. 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 study³. There have been several genome-wide linkage scans reporting suggestive linkage regions on several chromosomes, but only two genes - AGC1⁴⁻⁶ 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).

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.

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.

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}

We meta-analyzed 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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Figure legends:

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

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

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Supplementary methods

Study samples

The study participants were from five samples – the TwinsUK cohort and the Rotterdam discovery subset as discovery samples, the Chingford study, the Chuvashia Skeletal Aging study, the Rotterdam replication subset, and the GARP Study as replication samples. The characteristics of the cohorts are presented in table 1.

The TwinsUK cohort consisted of a group of twins ascertained to study the heritability and genetics of age-related diseases (www.twinsUK.ac.uk). These unselected twins were recruited from the general population through national media campaigns in the UK and shown to be comparable to age-matched population singletons in terms of disease-related and lifestyle characteristics¹. We examined a subcohort consisting of 799 women with both genotype and phenotype data available. Radiographs of both hands were obtained with a standard posteroanterior view. 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 (K/L) score using a standard atlas². All radiographs were independently assessed by two trained observers who were blind to the genotyping data, and in cases of disagreement a third adjudicator was used. The intraobserver and interobserver reproducibility of the scoring were tested on a subgroup of 50 hands with a (kappa) statistic of over 0.68 for all sites and features. We summed the KL score of each hand joint and adjusted for age and age square. The normalized residuals of the total KL score were used as quantitative measurement of hand OA in the genome-wide association analysis (GWAS).

The Rotterdam Study is a prospective population based investigation of the determinants and prognosis of chronic diseases in 7983 elderly people. The design of the study has been described elsewhere³. Written informed consent was obtained from each participant. The Rotterdam Study was approved by the medical ethics committee of Erasmus University Medical School. At the discovery stage, 1005 Dutch women from the Rotterdam Study were available. The remainder of the Rotterdam Study consisting of 557 men and 609 women were available for replication stage. Standard anteroposterior radiographs of both hands were scored by two trained assessors, who were blinded to the clinical and demographic data. Each joint was graded for overall ROA using a modified Kellgren–Lawrence (K-L) grade scaled from 0 to 4 as described before⁴. The normalized residuals of the total KL score after adjustment for age and age square was used in the replication analysis.

The Chingford Study is a well-described 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^{5,6}. Women from this practice are similar to women in the UK general population in terms of weight, height, and smoking characteristics⁵. Radiographs of both hands were obtained with a standard posteroanterior view and assessed in the same manner as in the TwinsUK cohort and by the same reader. The normalized residuals of the total KL score after adjustment for age and age square was used in the replication analysis.

The Chuvashia Study sample includes ethnical Chuvashians, who are a Caucasian Finno-Ugric speaking population residing in the Chuvasha and Bashkortostan autonomous regions of the Russian Federation. Each subject signed a written consent form containing information about the Chuvashian Skeletal Aging study. The study was approved by the Helsinki Ethics Committee of Tel-Aviv University, Tel Aviv, Israel. The individuals included in the present study were screened for known bone diseases and risk factors for increased bone loss (such as diabetes and hyperparathyroidism) and were naïve to common medications such as hormone replacement therapy and steroid medication. The present sample included in total 624 women, each of which was assessed for radiographic hand OA similar to the TwinsUK cohort^{6,12}. To assess the association we implemented sex –specific pedigree disequilibrium test, PDT⁶. The PDT examines the trait inheritance under the assumption that the marker locus itself is the gene controlling a part of the trait variation. The LRT is used to reject the null hypothesis that all marker genotypes exhibit the same mean trait value.

The GARP Study: The GARP study from Leiden, The Netherlands consists of 188 sibling pairs and 4 trios concordant for clinical and radiographically confirmed OA at two or more joint sites among hand, spine (cervical or lumbar), knee or hip⁷; random controls were partners of the offspring of the Leiden longevity study⁸. The association with the selected hand OA phenotype was assessed by linear mixed model analysis with the family identity numbers (representing family relations) as random variables in order to take into account the familial dependencies among sibling pairs. The association analyses were performed in 381 individuals of which genotypes and hand OA phenotypes were available.

Genotyping and quality control

For the TwinsUK discovery cohort, all samples were typed with the Infinium assay (Illumina, San Diego, USA) with fully compatible SNP arrays, the Hap300 Duo, Hap300, and Hap550. We pooled the normalised intensity data⁹ for 2820 Twins UK samples typed at Centre National de Génotypage, Duke University, NC, USA; Helsinki University, Finland; and the Wellcome Trust Sanger Institute, Cambridge, UK, and called genotypes on the basis of the Illuminus algorithm. No calls were assigned if the most likely call was less than a posterior probability of 0.95. Validation of pooling was done by visual inspection of 100 random, shared SNPs for overt batch effects; none were observed. 543 individuals were excluded because genotype concordance with another sample was more than 97% and the sample was of lesser call rate, or the sample call rate was less than 95%, or autosomal heterozygosity was more than 37% or less than 33%.

We excluded 3366 SNPs because $p \leq 1.0 \times 10^{-4}$ in test for deviation from Hardy–Weinberg equilibrium, or the minor allele frequency was 1% or less, or the call rate was 95% or less. We retained 305811 autosomal SNPs for analysis, with a resultant call rate of 99.3%. We also visually inspected all intensity cluster plots of SNPs that showed either an association for overdispersion of the clusters, biased no calling, or erroneous genotype assignment. We discarded SNPs with any of these characteristics.

Because of the relatedness of the TwinsUK cohort, we selected an independent sample from the cohort and assess potential population stratification. We used the whole genome-wide SNP data with reference to the HapMap three population data (CEU, YRI, and JPT+CHB) and calculated pair-wise probability of IBS using PLINK software¹⁰. We then used multidimensional scaling method to assess the population stratification. We also used the principal component method implemented in Goldsurfer software and self-reported ethnicity for confirmation. 51 individuals were identified as non-European origin and removed from the analysis.

The Rotterdam study samples were genotyped with the Infinium Hap550 assay. Intensity files were analysed with the Beadstudio Genotyping Module software v.3.1.14. A no-call threshold of 0.15 was applied to a custom-generated cluster file derived from the Illumina-provided cluster file. In the custom-cluster file 2308 SNPs with Genecall scores of less than 0.90 were visually checked by two observers and manually reclustered or zeroed accordingly. Samples with a low call rate and 10th percentile Genecall score were excluded before we called genotypes.

We excluded 209 samples with a call rate below 98%. 21 had heterozygosity rates above 37% or below 33% across all autosomal SNPs; six had ambiguous estimates of X chromosome inbreeding (homozygosity) ($0.2 < F < 0.8$); 36 had mismatch between called and phenotypic gender; 102 had outliers (3 SD) identified by the clustering analysis of identity by state; and 129 had outliers identified by identity-by-state probability of greater than 97%. In total, 706 samples were removed. The SNP quality control applied to the TwinsUK cohort was also applied to the Rotterdam cohort. After exclusions, 535 188 (95.3%) of all available SNPs were available for the replication analysis. We compared genotype accuracy against 22 Taqman SNPs, and recorded less than 0.3% discrepancy across genotyping methods.

For the Chingford Study and Chuvashia Study, All samples were carried out by Kbioscience, Hertfordshire, UK. SNPs were genotyped with the KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system using FRET quencher cassette oligos. Genotyping accuracy, as determined from the genotype concordance between duplicate samples, was 99.6%. The genotyping success rate was 97.9%. All polymorphisms were in Hardy Weinberg equilibrium in controls (all $p > 0.05$).

Genotypes of the GARP study and controls were performed by a fluorescent 5' exonuclease assay from a predesigned SNP TaqMan Genotyping Assay (Applied Biosystems, Foster City, CA). Genotyping quality was manually checked. The accuracy was determined from the 8-10% duplicate samples and was 100% and genotyping success rate was > 85%. The polymorphism was in Hardy Weinberg equilibrium in controls ($P = 0.5712$).

Statistical methods

To take into account of the relatedness in the TwinsUK cohort, we utilized the Merlin software package which is designed for GWAS analysis of family-based and twin data¹¹. The score test

implemented in the Merlin was used to test the association between a given SNP and the hand OA. We used log quantile-quantile (QQ) P-value plot to interpret the GWAS results. The negative logarithm of the i^{th} smallest P value is plotted against $-\log(i / (L+1))$, where L is the number of SNPs. Deviations from the $y=x$ line correspond to loci that deviate from the null hypothesis and therefore indicate a significant association. Linear regression modelling was used in the replication samples to examine the association between the selected SNPs and hand OA. Logistic regression model was also used to examine the association between the replicated SNP and hand OA in a case-control fashion.

To meta-analyze the summary results of the replicated SNP, we used a fixed effect model and inverse variance weighted average of β coefficients with STATA (StatCorp LP, College Station, TX, USA) and obtained a combined estimate of the overall β coefficient and its standard error. Between-study heterogeneity was assessed with the χ^2 test.

References

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Table 1. Characteristics of the study cohorts

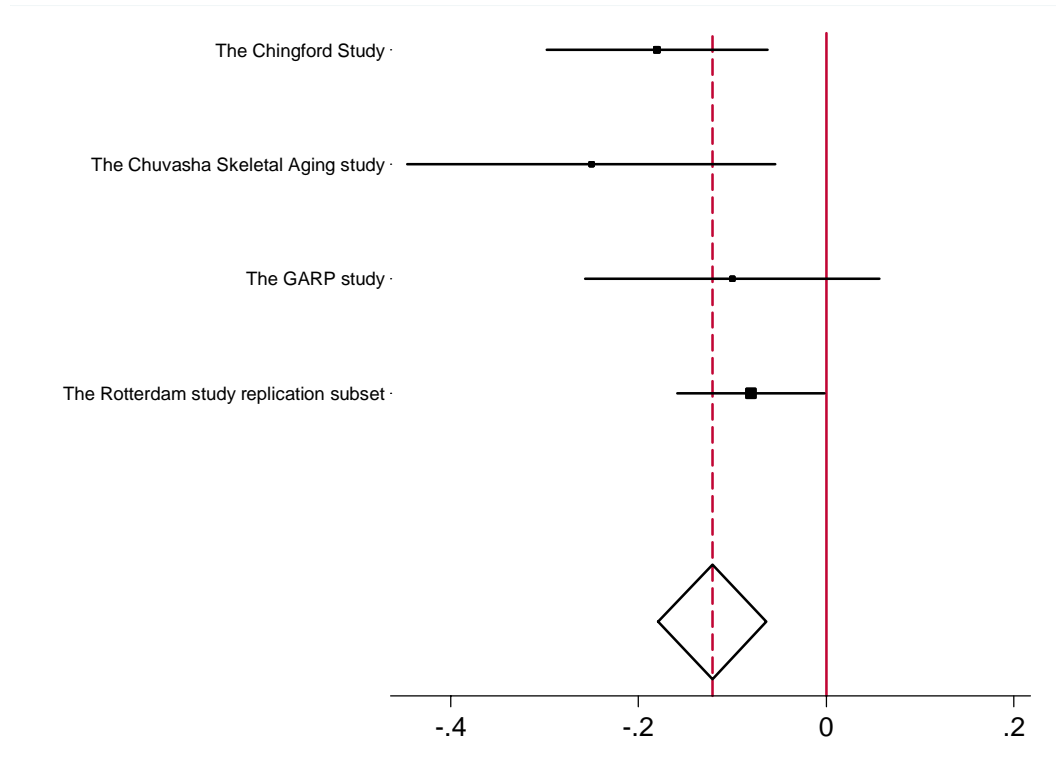
	The TwinsUK cohort (n=799)	The Rotterdam Study discovery subset (n=1005)	Chingford Study (n=637)	The Chuvashi a Study (n=624)	The GARP Study (n=381)	The Rotterdam Study replication cohort (n=1166)
females(%)	100%	100%	100%	100%	82%	52%
Age	54.28(7.83)	65.73(7.10)	54.68(6.02)	47.72(16.71)	60.26(7.55)	66.70(7.04)
Total KL score	4.71(7.75)	10.88(11.34)	6.15(8.35)	22.51(12.91)	16.8(13.82)	9.69(10.99)

Supplemental table: The top 100 SNPs associated with hand total KL score

CHR	SNP ID	Position	Minor Allele	MAF	β	SE	P value	P for HWE
1	rs549	15419412	A	0.472	0.209	0.055	0.0001596	0.6897049
1	rs12063364	31044638	G	0.295	-0.226	0.062	0.0002371	0.5536818
1	rs1334995	70899649	A	0.175	-0.27	0.073	0.0002291	0.2822353
1	rs2782763	70914790	C	0.166	-0.27	0.073	0.0002384	0.4893905
2	rs1473099	60303214	G	0.15	0.295	0.075	0.0000883	0.2137125
2	rs1922817	77566115	G	0.395	-0.202	0.056	0.0002798	0.1426987
2	rs4462808	81044720	T	0.433	0.214	0.056	0.0001311	0.4058234
2	rs4389353	81055194	T	0.433	0.195	0.054	0.0003366	0.5743892
2	rs13013804	211469823	G	0.127	0.315	0.085	0.0001942	0.724562
3	rs2124811	6453949	G	0.451	0.209	0.054	0.0001114	0.9367868
3	rs959132	87936967	C	0.408	0.208	0.054	0.0001101	0.4669694
3	rs2904196	118829308	T	0.244	0.216	0.06	0.0003541	0.0278494
3	rs10513209	144752348	T	0.133	0.333	0.078	0.0000224	0.7432328
3	rs17582102	144794143	C	0.14	0.277	0.078	0.0003612	0.8719814
3	rs6443283	176302889	T	0.124	0.291	0.081	0.0003444	0.2329225
3	rs9879640	179480262	G	0.462	0.225	0.057	0.0000729	0.6815721
4	rs11731332	6164460	C	0.459	0.201	0.055	0.0002671	0.8751011
4	rs11940311	76321422	T	0.273	0.233	0.063	0.0002316	0.0898901
4	rs7691721	76383311	C	0.285	0.247	0.062	0.0000708	0.1446831
4	rs10030550	80114347	G	0.147	0.295	0.08	0.0002307	0.0230402
4	rs10049840	80551063	A	0.275	0.221	0.061	0.0002796	0.9223238

5	rs2443547	18209429	C	0.482	0.198	0.056	0.0003573	0.3886167
5	rs442958	18212340	A	0.483	0.199	0.056	0.0003358	0.3897962
5	rs10057505	65185571	G	0.486	-0.195	0.054	0.000279	0.6386093
5	rs11948927	65195821	C	0.42	0.193	0.054	0.0003557	0.2902385
5	rs7356540	91339475	C	0.176	0.25	0.07	0.0003398	0.3372491
5	rs2112945	91358638	G	0.176	0.259	0.07	0.0001937	0.3359232
5	rs13162151	124032303	A	0.133	0.276	0.076	0.0003037	0.5240533
5	rs32218	127719132	G	0.167	0.313	0.074	0.0000243	0.2627492
5	rs7720295	163315253	T	0.282	0.236	0.061	0.0001041	0.0928089
6	rs9442813	73301649	A	0.449	-0.21	0.056	0.0001581	0.4289273
6	rs6921577	167003680	G	0.45	0.21	0.054	0.0001094	1
6	rs12213619	167698488	G	0.286	0.238	0.06	0.0000788	0.6398427
7	rs1447398	4519485	G	0.283	0.217	0.06	0.0002687	0.9265906
7	rs1522919	29112231	G	0.129	-0.319	0.088	0.0002936	0.3187056
7	rs1034716	29400969	G	0.366	0.233	0.056	0.0000315	0.6703193
7	rs6949736	48330883	A	0.424	0.211	0.058	0.0002836	0.0085711
7	rs6465748	98699243	T	0.151	0.271	0.072	0.0001845	0.3429512
7	rs2037595	98923815	A	0.158	0.298	0.068	0.000013	0.7009723
7	rs11772856	115822080	C	0.369	0.214	0.057	0.0001597	1
8	rs13265778	58840748	T	0.192	0.322	0.071	5.76E-06	0.3083875
8	rs1561081	58856665	A	0.125	0.349	0.084	0.0000318	0.0856192
8	rs11989824	72812394	T	0.275	0.238	0.062	0.0001067	0.8447989
8	rs7813226	123518131	C	0.17	0.281	0.071	0.0000668	0.5098361
9	rs3758201	80108240	C	0.369	0.207	0.057	0.0002795	0.6707519
9	rs10867419	81551987	C	0.297	-0.216	0.059	0.000233	0.1548232
9	rs1888073	81567263	T	0.257	-0.236	0.061	0.0001214	0.1179802
9	rs2170226	115503981	T	0.165	0.271	0.074	0.0002653	0.6723067
9	rs1465918	118612697	G	0.189	0.251	0.07	0.0003161	1
9	rs445990	134473542	T	0.489	0.219	0.056	0.0000993	0.1263075
10	rs7912487	3194561	C	0.266	0.221	0.062	0.0003204	0.3206448
11	rs4756876	16920239	C	0.112	0.432	0.092	2.35E-06	0.0672135
11	rs7107938	27027694	T	0.479	-0.197	0.053	0.0002024	0.2720307
11	rs4939346	59879433	A	0.31	-0.235	0.063	0.000205	0.4203446
11	rs1430860	94111575	G	0.172	0.274	0.068	0.0000564	0.0228369
11	rs7947141	109370769	C	0.144	-0.29	0.08	0.0003038	0.8609972
11	rs4146886	114423137	G	0.227	0.244	0.066	0.0001984	0.5622515
11	rs948725	123915145	G	0.245	-0.245	0.064	0.0001166	0.9142427
11	rs2187149	123937533	A	0.303	-0.212	0.058	0.0002574	0.7152309
11	rs12792184	123945827	C	0.245	-0.255	0.063	0.0000594	0.8301603
11	rs2063071	132260488	A	0.371	-0.203	0.055	0.0002304	0.1719446
12	rs7970960	17993577	C	0.137	0.289	0.079	0.000259	0.6287886
12	rs7305652	43311663	T	0.116	0.302	0.085	0.0003659	0.7134909

12	rs10506257	43444556	T	0.266	0.247	0.059	0.0000307	0.0480756
12	rs7311560	75129049	T	0.184	0.268	0.072	0.0002004	0.7890555
12	rs4882730	127358334	A	0.125	0.32	0.085	0.0001733	1
13	rs12871898	24774011	G	0.469	0.214	0.055	0.0000901	0.7536178
13	rs1873288	84779125	G	0.19	0.238	0.064	0.0001991	0.1907894
14	rs1958654	53298267	T	0.143	0.296	0.081	0.0002462	0.1891808
14	rs979536	70379236	A	0.125	0.353	0.086	0.0000377	0.0997245
14	rs1990037	70384334	C	0.123	0.349	0.086	0.0000495	0.1372565
14	rs11623996	70807067	G	0.226	0.252	0.063	0.0000555	0.5231641
14	rs41216	70921665	C	0.32	0.22	0.058	0.0001474	0.6624764
15	rs938076	23784012	A	0.455	0.22	0.053	0.0000372	0.4323534
15	rs12901404	31862592	C	0.122	0.431	0.087	7.78E-07	0.2771535
15	rs12900756	31876788	G	0.16	0.272	0.076	0.0003624	0.1865962
15	rs1055356	32935394	T	0.427	-0.226	0.054	0.000033	0.8738328
15	rs3858931	32939941	T	0.395	-0.199	0.055	0.0003308	0.9353293
15	rs717941	32984170	A	0.396	-0.208	0.055	0.0001632	0.6847224
15	rs7169701	33004828	T	0.397	-0.202	0.055	0.0002467	0.8080193
16	rs716508	6276913	C	0.313	-0.24	0.06	0.000064	0.3186542
16	rs4786850	6285925	C	0.279	-0.243	0.062	0.0000839	0.2473599
17	rs7217186	4486141	C	0.469	0.212	0.056	0.0001405	0.6383255
17	rs4794067	43163827	C	0.271	-0.226	0.061	0.0002216	0.3246612
17	rs8081512	62139328	A	0.465	0.197	0.054	0.0003065	1
17	rs1529964	73217934	C	0.345	0.22	0.059	0.000201	0.1356426
18	rs1985049	51784784	T	0.112	0.314	0.085	0.0002049	1
18	rs2654179	73604134	C	0.411	-0.201	0.055	0.0002891	0.7447182
18	rs1032914	73628654	C	0.41	0.222	0.054	0.0000336	0.3024007
18	rs4890850	73641146	T	0.417	-0.207	0.056	0.000221	0.6267223
18	rs7241566	73676037	T	0.4	0.222	0.054	0.000039	0.6312391
18	rs660749	75456991	C	0.409	0.199	0.056	0.0003452	0.6868778
19	rs731945	18428039	T	0.295	0.236	0.06	0.0000772	0.6423206
19	rs879122	34447521	T	0.239	0.242	0.063	0.0001238	0.8316381
19	rs281377	53898415	T	0.443	0.216	0.055	0.0000955	0.2660386
20	rs6109781	13181011	C	0.166	0.26	0.073	0.0003596	0.1237613
20	rs6092326	54550144	C	0.485	0.225	0.054	0.0000277	0.2720564
20	rs4811713	54565885	C	0.351	0.24	0.056	0.0000195	0.6698828
21	rs7277441	32363481	C	0.493	-0.188	0.053	0.0003632	0.1340927
22	rs5767675	46089479	G	0.366	-0.224	0.06	0.0002075	0.1333105

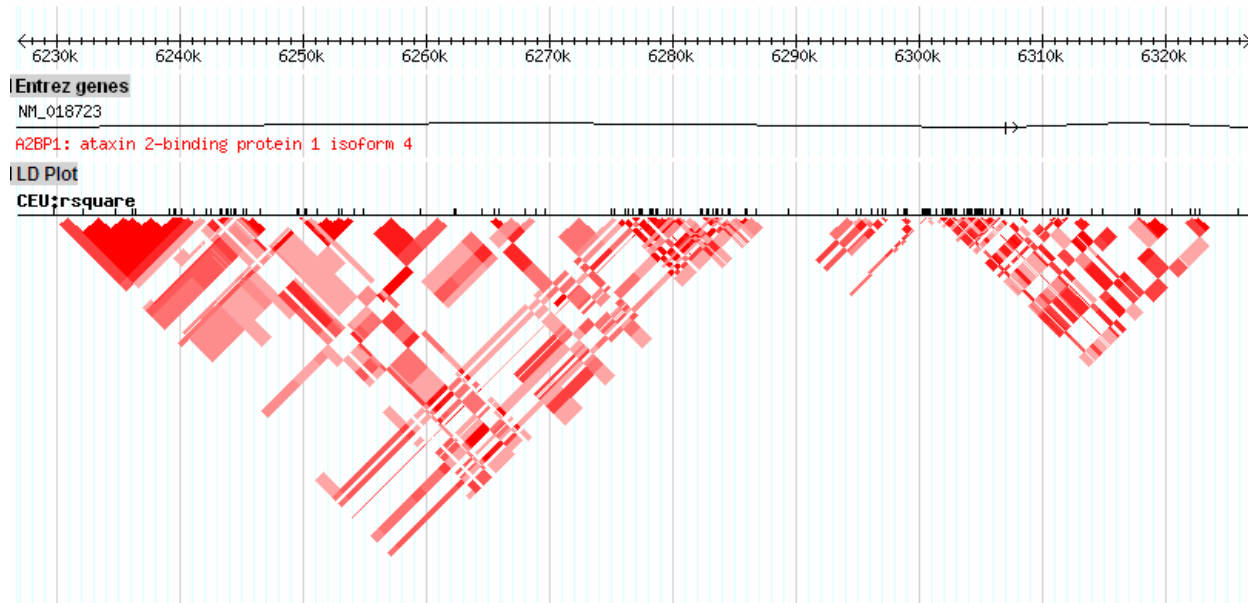


Supplemental Figure 1. Forrest plot for the meta-analysis with replication cohorts.

Supplemental Figure 2

Linkage disequilibrium plot: (A) Plot of 100 kb genomic region of A2BP1 gene with the association SNP (rs716508), SNPs genotyped in HapMap, and linkage disequilibrium among SNPs (r^2 is shown). r^2 values of 1.0 are depicted by red diamonds, intermediate r^2 values are represented in pink, and r^2 values of 0 as white. (B). Plot of LD block containing the association SNP rs716508. The values on the diamonds are r^2 . rs716508 is low LD with flanking SNPs in the LD block.

A. chr16: 6226913 - 6326912



B. chr16: 6276441 – 6278222

