A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip.

Supplementary Material

Content

1. Supplementary Methods

a. Genotyping, data quality control and data imputation

To allow for meta-analysis across different marker sets, imputation of polymorphic HapMap European CEU SNPs was performed using MACH or IMPUTE [1]. Two research centres (Ioannina, Greece and Erasmus MC Rotterdam, the Netherlands) performed both the Quality Control (QC) and meta-analyses. A QC protocol was set up including validation of the results file format, reports for range of values and elimination of potential biases (i.e., extremely large beta's or SEs). Files were cross-validated between the two research centers after QC and after meta-analyses to check for inconsistencies. SNPs with a MAF <1%, imputation quality <0.30 (MACH) or <0.40 (IMPUTE) and beta's >4 or <-4 were excluded for further analysis.

b. Statistical analysis

The principal summary measure of association was the per-allele odds ratio (OR). We performed genomic control at the individual study level estimates; for each study, we recorded the inflation factor lambda for the study so as to adjust the standard error of the effect size (standard error is multiplied by the square root of lambda).

We summarized OR estimates using fixed-effects models [2]. Fixed-effects models assume that there is a common underlying effect and the variability observed is attributed to chance alone; random effects models acknowledge that true between-study heterogeneity exists, take into account the presence of heterogeneity into their calculations and, in the presence of heterogeneity, yield more conservative estimates. In the absence of heterogeneity, fixed- and random-effects models yield the same results. Fixed-effects models are more appropriate at the SNP discovery and prioritization stage and perform well at the replication stage. The presence of statistically significant heterogeneity was assessed by the Q statistic (significant at $p < 0.10$) and the extent of the observed heterogeneity was assessed by the I^2 (ranging from 0% to 100%) [3]. We also summarized OR estimates under a random-effects model proposed by Han and Eskin [4].

c. Study participants

The Rotterdam Study I, II, & III: The study population comprises men and women aged 55 years and older of the Rotterdam Study, which is a prospective population-based study on determinants of chronic disabling diseases. It consists of three sub-popualtions and the rationale and study design have been described previously [5]. The medical ethics committee of Erasmus University Medical School approved the study and written informed consent was obtained from each participant. Hip OA cases were defined as a KL grade≥2 or total hip replacement.

deCODE: a list of patients with OA of hip was obtained on the basis of patients' records at hospitals and health care centers in Iceland [6]. Controls were individuals with no external signs of OA in any joint who did not have a diagnosis of primary OA. The study was approved by the Data Protection Authority of Iceland and the National Bioethics Committee of Iceland. Informed consent was obtained from all participants.

TwinsUK: the study participants were white monozygotic and dizygotic twin pairs from the TwinsUK adult twin registry, a group used to study the heritability and genetics of age-related diseases [7]. These unselected twins were recruited from the general population through national media campaigns in the United Kingdom. Ethics approval was obtained from the Guy's and St. Thomas' Hospital Ethics Committee. Written informed consent was obtained from every participant.

The Genetics OsteoArthritis and Progression (GARP) study from Leiden, the Netherlands, consisted of 192 sibling pairs concordant for clinical and radiographically (K/L score) confirmed OA at two or more joint sites among hand, spine (cervical or lumbar), knee or hip [8], random controls (N=758) were partners of the offspring of the Leiden longevity study [9]. To comply with the discovery sample OA phenotypes for knee, hip and hand OA used were based on radiographic signs OA Written informed consent was obtained from each subject as approved by the ethical committees of the Leiden University Medical Center.

arcOGEN study

arcOGEN stage 1: The arcOGEN case samples were collected in two stages. The stage 1 samples comprised 1,728 hip cases from existing DNA collections from five United Kingdom locations within the arcOGEN consortium (London, Nottingham, Oxford, Sheffield, and Southampton). The detailed characteristics of these cases are described elsewhere [10]. Briefly, all were unrelated and of European origin, and all had primary OA of the hip of radiographic Kellgren-Lawrence (KL) grade ≥2, or clinical evidence of disease to a level requiring total joint replacement (TJR). The stage 1 study used 4,894 population-based UK controls from an early release of the Wellcome Trust Case Control Consortium 2 (WTCCC2) data which came from 2 distinct sources: the 1958 Birth Cohort [58BC] and the UK Blood Donor Service (UKBS) and were unrelated (www.wtccc.org.uk).

arcOGEN stage 2: The stage 2 cases (n=1,763 with hip OA) were collected prospectively as part of the arcOGEN study at nine locations across the UK (Edinburgh, London, Newcastle-Upon-Tyne, Nottingham, Oxford, Sheffield, Southampton, Wansbeck, and Worcester) [11]. The ascertainment criterion was primary OA that was severe enough for the individual to require joint replacement of the hip. All cases were unrelated and of European origin. The controls (n=6,157) were population-based, unrelated UK controls which came from five distinct sources: the 1958 Birth Cohort from the Type 1 Diabetes Genetics Consortium (T1DGC) study, the Avon Longitudinal Study of Parents

and Children (ALSPAC), the People of the British Isles (PoBI) study and additional controls from the 58BC and the UKBS from the WTCCC2 study that were not overlapping with those used in stage 1.

arcOGEN plus: The arcOGEN plus dataset (n=223 females with hip OA) comprises additional cases collected in stage 2 which were genotyped at a later stage. The ascertainment criterion was primary OA that was severe enough for the individual to require joint replacement of the hip. Controls (n=1,828) were unrelated, OA-free controls (females only) from the TwinsUK cohort which consist of twins ascertained to study the heritability and genetics of age-related diseases (www.twinsUK.ac.uk). Samples that overlapped with the TwinsUK dataset used in the discovery analysis were excluded from this study.

Estonian Genome Center, University of Tartu (EGCUT). The Estonian cohort is from the population based biobank of the Estonian Genome Project of University of Tartu. The whole project is conducted according to the Estonian Gene Research Act and all participants have signed the broad inform consent. The current cohort size is over 51,515, from 18 years of age and over, which reflects closely the age distribution of the adult Estonian population. Subjects were recruited randomly when visiting general practitioners (GPs) and hospitals. Each participant filled out a Computer Assisted Personal interview during 1-2 hours at doctors; office, including personal data. OA was diagnosed by a specialist as a clinical finding and was usually confirmed by a radiograph (KL grade>2). The OA cases for the current study had an ICD10 M16 and/or M17 diagnosis.

Greek case-control study: The individuals included in the study were of Greek origin living in the district of Thessalia in central Greece [12]. All of them had undergone a TKR/THR, meaning that all of them suffered from severe knee or hip OA, which is defined by a K/L grade >=2. None of the patients had evidence of arthritis due to another disease. All the controls had a K/L score of 0 and had undergone treatment for injuries or fractures. Patients with rheumatoid arthritis and other autoimmune diseases as well asmchondrodysplasias, infection-induced OA, and posttraumatic OA were not included in the study. The ethics committee of the Larissa University Hospital approved this study and all individuals gave their informed consent.

Spanish TJR cases: Patients were selected from consecutive patients, aged 55-75 years of age at time of the surgery, undergoing THR/TKR [13]. All patients were included if a rheumatologist considered them to suffer from severe primary OA. Exclusion criteria were inflammatory, infectious, traumatic or congenital joint pathology and lesions due to crystal deposition or osteonecrosis. Controls were recruited among subjects older than 55 years of age undergoing preoperative work-up for elective surgeries other than joint surgery and who did not show clinical manifestations of OA. This study was approved by the Ethical Committee for Clinical Research of Galicia and all cases and controls gave their written informed consent to participate.

Swedish Malmo Diet and Cancer (MDC) study: All men and women living in the city of Malmö in Sweden, who were born between 1923 and 1945 (men) or between 1923 and 1950 (women) were invited to participate in the Malmö Diet and Cancer (MDC) study. The screening examination was performed during 1991-1996. All participants (n=28449) were followed until first OA surgery, emigration from Sweden, death or December 31 2005, whichever came first. Hip osteoarthritis was defined as a first hip arthroplasty (procedures coded 8414, 8010, NFB09, NFB19, NFB29, NFB39, NFB49 and NFB99) in combination with a contemporaneous diagnosis of hip osteoarthritis (715 or M16 according to ICD-9 and ICD-10, respectively). Cases were matched (1:1) for age, gender and BMI, to MDC participants without THR in a nested case-control design.

Osteoporotic Fractures in Men Study (MrOS): The Osteoporotic Fractures in Men Study (MrOS) is a multi-center prospective, longitudinal, observational study of risk factors for vertebral and all non-vertebral fractures in older men, and of the sequelae of fractures in men [14,15]. The original specific aims of the study include: (1) to define the skeletal determinants of fracture risk in older men, (2) to define lifestyle and medical factors related to fracture risk, (3) to establish the contribution of fall frequency to fracture risk in older men, (4) to determine to what extent androgen and estrogen concentrations influence fracture risk, (5) to examine the effects of fractures on quality of life, (6) to identify sex differences in the predictors and outcomes of fracture, (7) to collect and store serum, urine and DNA for future analyses as directed by emerging evidence in the fields of aging and skeletal health, and (8) define the extent to which bone mass/fracture risk and prostate diseases are linked. The MrOS study population consists of 5,994 community dwelling, ambulatory men aged 65 years or older from six communities in the United States (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley near Pittsburgh, PA; Portland, OR; and San Diego, CA). Inclusion criteria were designed to provide a study cohort that is representative of the broad population of older men. The inclusion criteria were: (1) ability to walk without the assistance of another, (2) absence of bilateral hip replacements, (3) ability to provide self-reported data, (4) residence near a clinical site for the duration of the study, (5) absence of a medical condition that (in the judgment of the investigator) would result in imminent death, (6) ability to understand and sign an informed consent, and (7) 65 years or older. To qualify as an enrollee, the participant had to provide written informed consent, complete the self-administered questionnaire (SAQ), attend the clinic visit, and complete at least the anthropometric, DEXA, and vertebral X-ray procedures. The MrOS cohort recruited only men.

Study of Osteoporotic Fractures (SOF): The Study of Osteoporotic Fractures (SOF) is a prospective multicenter study of risk factors for vertebral and non vertebral fractures[16]. The cohort is comprised of 9704 community – dwelling women 65 years old or older recruited from populations-based listings in four U.S. areas: Baltimore, Maryland; Minneapolis, Minnesota; Portland, Oregon; and the Monongahela Valley, Pennsylvania. Women enrolled in the study were 99% Caucasian with African American women initially excluded from the study due to their low incidence of hip fractures. A cohort of AA women was recruited at the 6th Visit. The SOF participants were followed up every four months by postcard or telephone to ascertain the occurrence of falls, fractures and changes in address. To date, follow-up rates have exceeded 95% for vital status and fractures. All fractures are validated by x-ray reports or, in the case of most hip fractures, a review of pre-operative radiographs. The inclusion criteria were: 1) 65 years or older, (2) ability to walk without the assistance of another, (3) absence of bilateral hip replacements, (4) ability to provide self-reported data, (5) residence near a clinical site for the duration of the study, (6) absence of a medical condition that (in the judgment of the investigator) would result in imminent death, and (7) ability to understand and sign an informed consent. To qualify as an enrollee, the participant had to provide written informed consent, complete the self-administered questionnaire (SAQ), attend the clinic visit, and complete at least the anthropometric measures. The SOF study recruited only women

Paprika study: The Paprika study is performed at the Leiden University Medical Center (Dept. Orthopedics) and consists in a long-term follow-up study of patients that have undergone total joint replacement (TJR) at hip or knee [17-19] and has been approved by the medical ethical committee. Patients of Caucasian descent were included when they were diagnosed with primary osteoarthritis based on radiographs and the ACR rheumatology classification criteria (mean age males-hip: 66; years males-knee: 68 years; females-hip: 66 years; females-knee: 69 years). Patients

with secondary OA or requiring a revision were excluded in this study. Written consent was obtained from each participant.

Genotyping.

The Rotterdam Study I, II & III: Genotyping of the samples with the Illumina HumanHap550v3 Genotyping BeadChip was carried out at the Genetic Laboratory of the Department of Internal Medicine of Erasmus Medical Center, Rotterdam, the Netherlands. The Beadstudio GenCall algorithm was used for genotype calling and quality control procedures were as described previously [20]. The following quality control filters were applied: SNP call rate >= 95%, minor allele frequency >= 5%, p-value HWE >=1x10-6. After quality control 500,510 SNPs remained for association analyses. The intensity cluster plots were visually inspected for the top-hits of the Rotterdam Study and no abnormalities were discovered. Genomic inflation factors were calculated for all analyses and there was no evidence of population stratification with lambdas of 1.01 for hip- and hand-OA, 1.00 for knee-OA

deCODE: All samples were assayed with the Infinium HumanHap 300 or humanCNV370 SNP chips (Illumina), containing 317,503 tagging SNPs derived from phase I of the International HapMap project. All of the SNPs tested in this report passed quality filtering (a call rate >97%, a minor allele frequency >1%, not a significant distortion from HWE (p-value >10-7 on any of the three chip types used (humanHap300, humanHap300-duo and humanCNV370). Any samples with a yield <98% were excluded from the analysis. Imputation was done using the IMPUTE software [1]. The additional cases in the replication analysis were genotyped using the Centaurus (Nanogen) platform

TwinsUK: Samples were genotyped with the Infinium HumanHap 300 assay (Illumina, San Diego, USA) at the Duke University Genotyping Center (NC USA), Helsinki University (Finland) and the Wellcome Trust Sanger Institute. The Illuminus calling algorithm was used for genotype calling. After strict quality control criteria were applied as described in [20] there were 314075 SNPs available for analysis. Imputation was performed using the IMPUTE software (v0.2.0) [1]. At imputed loci, all genotypes with posterior probabilities < 0.9 were discarded and the imputed loci were filtered out using usual QC filters.

Genetics OsteoArthritis and Progression (GARP) Study: For the GARP study the genome wide scan was genotyped by Illumina Infinuum II HumanHap 55KL Beadchips and Illumina Infinium II HumanHap550-Duo BeadChips (Illumina, San Diego, USA), respectively. Genotypes from the SNPs from the HapMap phase II v21 were imputed using IMPUTE.

arcOGEN study: arcOGEN stage 1 and stage 2 cases were genotyped using Illumina Human 610-Quad BeadChips. The publically available controls used for stage 1 and for stage 2 were genotyped on a variety of platforms (Table 1) [10,11]. ArcOGEN plus cases were genotyped on the Illumina HumanOmniExpress platform. This study used TwinsUK disease-free controls which were genotyped on Illumina Human 610-Quad BeadChips. All datasets underwent QC at the sample and SNP level separately for each case and control cohorts as previously [10,11]. Briefly samples were excluded if their call rate was <97% and if they showed gender discrepancies (estimated from genotypic data against external information). Individuals were also excluded on the basis of excess genome-wide heterozygosity or homozygosity. We identified samples that were accidentally duplicated or closely-related by calculating genome-wide IBD (given IBS information) for pairs of individuals. Multidimensional scaling (MDS) was performed in conjunction with data from the three HapMap phase II populations in order to identify and exclude individuals of non-

European descent. SNPs were excluded from further analysis based on the following criteria: Call rate <95% if minor allele frequency (MAF)≥5% or call rate <99% if MAF<5%, HWE exact p values <0.0001 in cases or controls, and MAF <1%. Association analyses were carried out under the additive model. Imputation was carried out using IMPUTE and imputed genotypes were analysed taking under account the full genotype probability distribution.

Osteoporotic Fractures in Men Study (MrOS) and Study of Osteoporotic Fractures (SOF): The Illumina HumanOmni1_Quad_v1-0 B was used for whole-genome genotyping. Samples from SOF and MrOS were randomized to 96-well genotyping plates by sex and clinic site. Eighty-one samples were plated twice to assess reproducibility. Pairwise concordance was 100%. 119 replicates of samples from HapMap trios of CEU and YRI populations and singletons from CHB and JPT populations were genotyped alongside MrOS and SOF samples, and compared to published HapMap genotypes. Concordance was 99.7% for CEU and YRI samples and was 95.0-99.7% for CHB and JPT samples. Genotypes were called using a clustering algorithm in Illumina's BeadStudio software at the Broad Institute. Samples with call rates < 97% were excluded. SNPs with GenTrain scores <0.6, cluster separation scores <0.4, call rates <97%, or MAF <0.01 were excluded. Autosomal SNPs with HWE P-value $<10^{-4}$ were excluded. In addition, genotype clusters for SNPs on chrX, chrY, chrXY and chrMT were reviewed manually. For MrOS and SOF samples, 740,713 SNPs passed QC.Additional samples were excluded based on: (1) genotypic sex mismatch using X and Y chromosome probe intensities, (2) relatedness among genotyped samples using the kinship coefficient that estimates probability that alleles are identical-by-descent, and (3) gross chromosomal abnormalities detected using the LogR Ratio and B allele frequency. Among the 3924 SOF samples that underwent whole-genome genotyping, 3682 samples had acceptable call rates. Among these 3682 SOF samples, 4 were removed due to relatedness and 53 were removed due to gross chromosomal abnormalities, leaving 3625 SOF samples with whole genome genotyping data that passed QC. Among the 5506 MrOS samples that underwent whole-genome genotyping, 5189 samples had acceptable call rates. Among these 5189 MrOS samples, 1 was removed due to relatedness and 37 were removed due to gross chromosomal abnormalities, leaving 5151 MrOS samples with whole genome genotyping data that passed QC. SNPs and samples that passed QC filters underwent SNP genotype imputation using minimac. HapMap phase II release 22 build 36 consensus phased haplotypes from a combined panel of CEU, YRI, CHB, and JPT HapMap samples were used as a reference panel

Estonian Genome Center, University of Tartu (EGCUT). All samples were genotyped with Illumina HumanCNV370 or HumanOmniExpress (Illumina, San Diego, USA) according to the Illumina protocol in the Estonian Biocenter. Data quality control was perform with PLINK (SNP call rate>98%;sample call rate>95%; MAF>0.01; HWE $p>10^{-6}$; cryptic relatedness). Imputation was performed with IMPUTE v1.0 (CEU HapMap rel22 build 36) and association analyses were carried out with SNPTEST. Inflation factors for directly genotyped and imputed data were 1.01 and 1.01 respectively.

Paprika study: In the present work, genotyping of the Paprika study was performed using the Sequenom MassARRAY iPLEX Gold or Taq-Man SNP Genotyping assays following the manufacturer's instructions. All SNPs passed the following quality criteria: call rate >98% and p-value for Hardy-Weinberg equilibrium <10-4.

d. De novo genotyping for imputed SNPs rs6094710 and rs640070

Imputed SNPs rs6094710 and rs640070 had MAF <4% and even though they passed the imputation quality criteria set upfront therefore they were de novo genotyped to minimize the possibility of imputation errors. Random samples of three populations from TWINS UK (n=392), arcOGEN (n=1046) and Rotterdam (n=865) were used for the assessment. For rs6094710 the concordance was 97.7%, 98.9% and 99.1% respectively. Poor concordance was found for rs640070 (<60% in all cases) and therefore it was excluded from further consideration.

e. Heritability of the identified markers of hip OA

We searched Pubmed for variants that have been identified as susceptible for hip OA in European populations. Only articles in English were eligible. We retrieved the hits that were GWS ($P<5x10^{-8}$) or reported as suggestive signals by the authors of the studies. From each study we recorded the study, the eligible variant, the risk allele frequency and the OR. We then calculated the sibling recurrence risk ratio attributed to these markers by using the formula $\lambda_s = \left(1 + \frac{pq(\gamma-1)^2}{2(p+\gamma q)^2}\right)^2$ where q is the risk allele frequency, p=1-q, γ=genotype relative risk under the log-additive model. The expected genetic variance explained was calculated as described in Ju-Hyun P et al [21]

2. Supplementary figures

FIGURE LEGENDS

Figure S1: Manhattan plot for the combined analysis of the hip OA GWAs meta-analysis.

Figure S2: QQ plot for the combined analysis of the hip OA GWAs meta-analysis. The expected p-value is indicated by the solid line and the associated 95% confidence intervals are indicated by the blue area either side

Figure S3: QQ plot for the A) female-specific and B) male-specific analysis of the hip OA GWAs meta-analysis. The expected p-value is indicated by the solid line and the associated 95% confidence intervals are indicated by the blue area either side

Figures S4-S11: Forest plots for the 8 SNPs that were followed-up in the 2nd stage of the analysis. The blue diamond denotes the summary effect size and its edges the respective 95% confidence intervals. Studies shaded in blue were included in the replication stage. * Discovery and replication estimate combined.

Figure S1

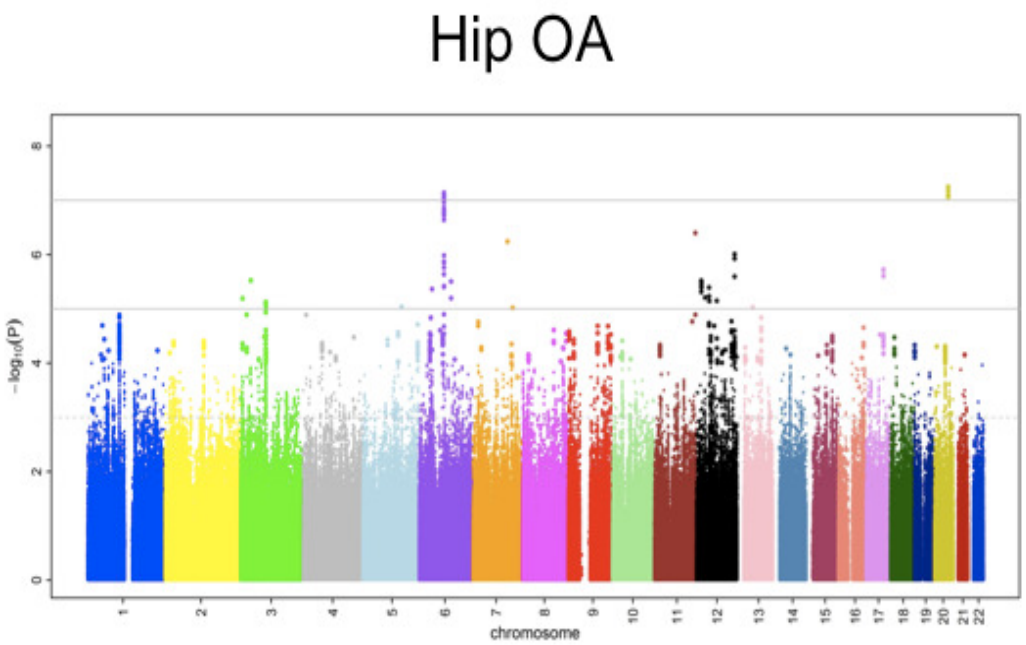


Figure S2

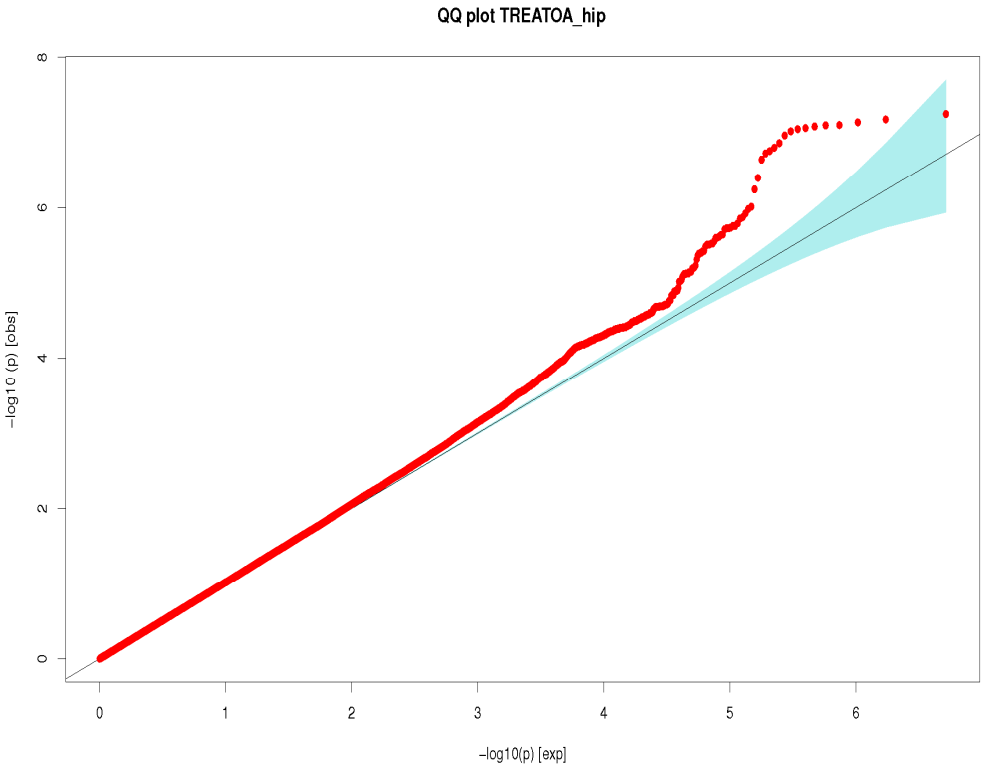


Figure S3

A)


QQ plot TREATOA_hip_females
−log10 (p) [obs]
−log10(p) [exp]
0
2
4
6
8
0
1
2
3
4
5
6

B)

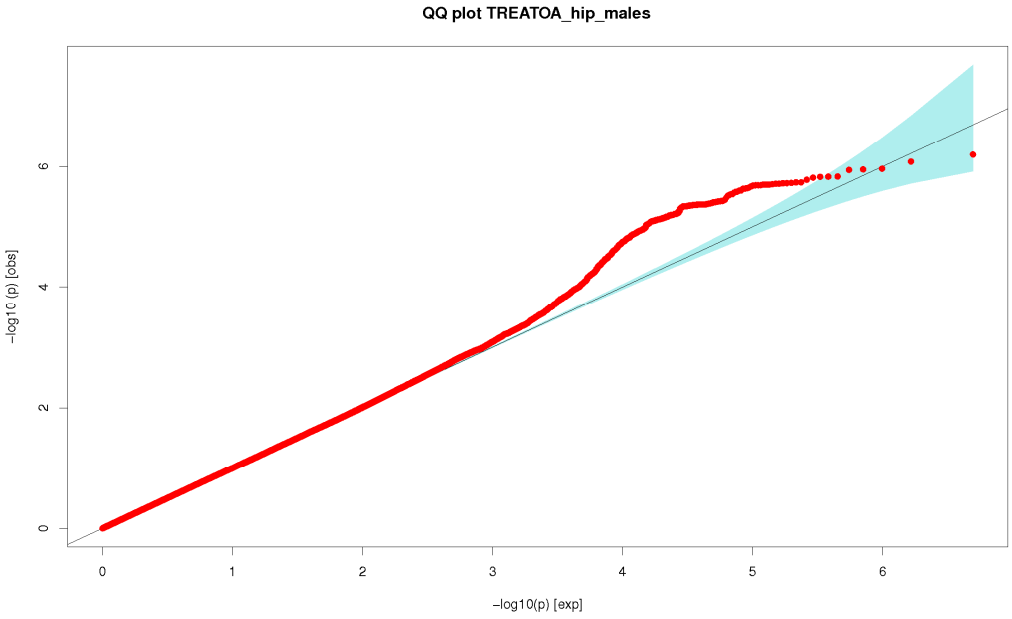

QQ plot TREATOA_hip_males
−log10 (p) [obs]
−log10(p) [exp]
0
2
4
6
0
1
2
3
4
5
6

Figure S4

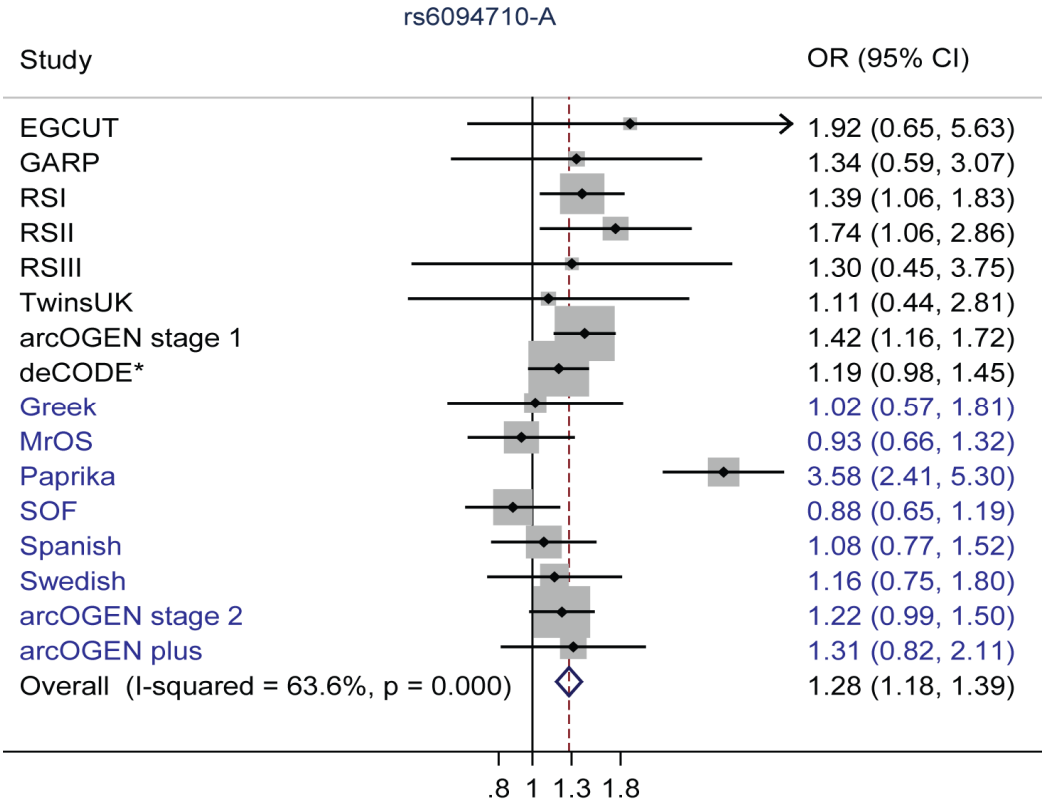


Figure S5

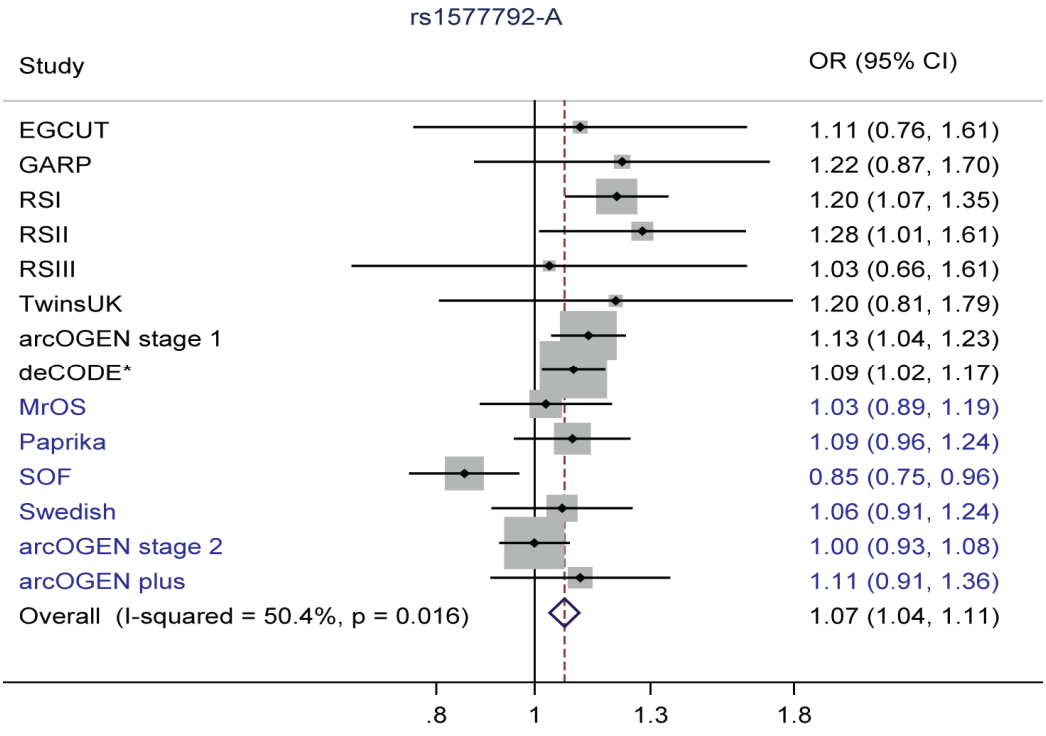


Figure S6

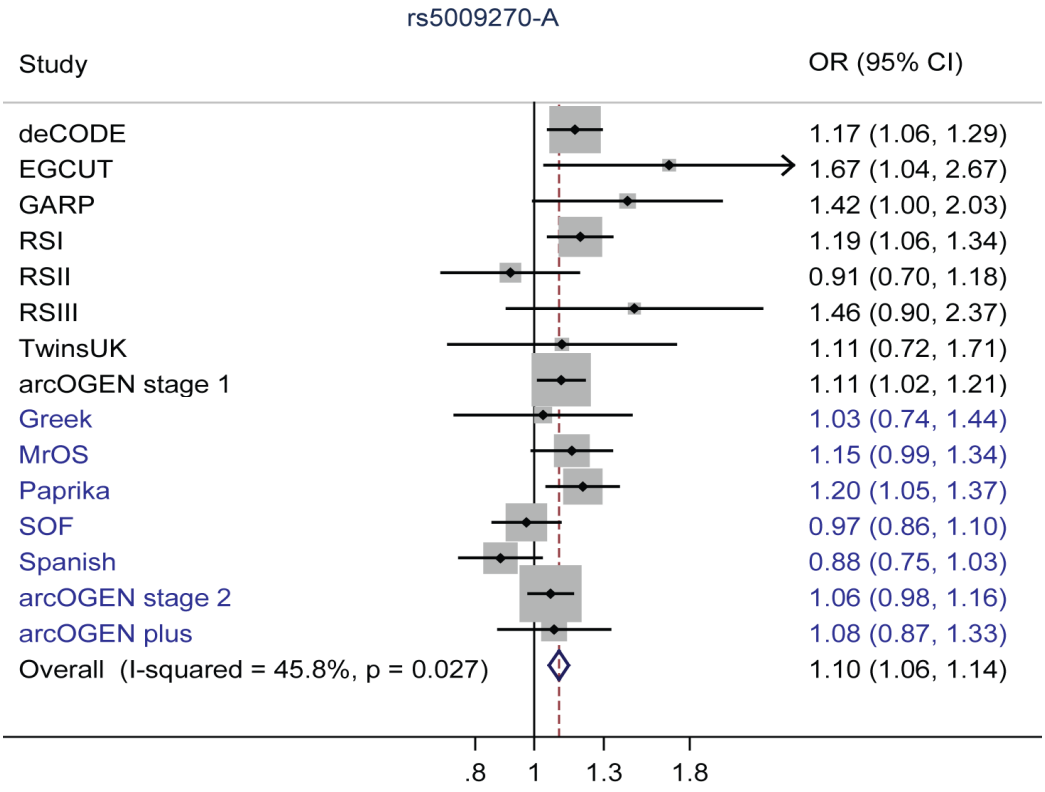


Figure S7

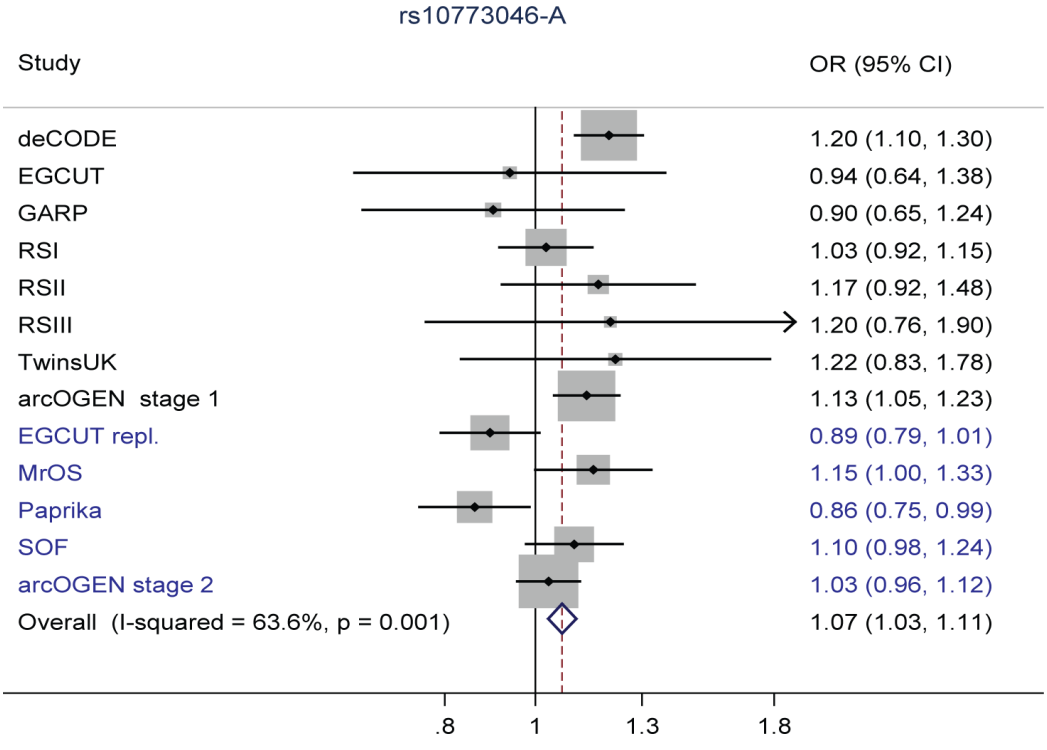


Figure S8

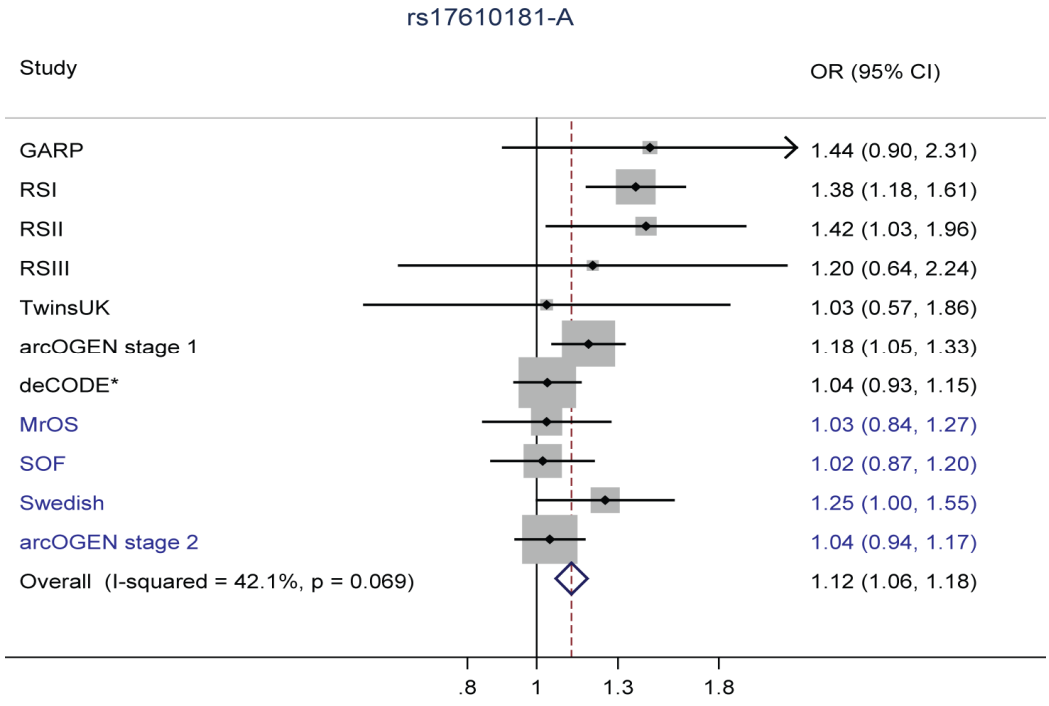


Figure S9

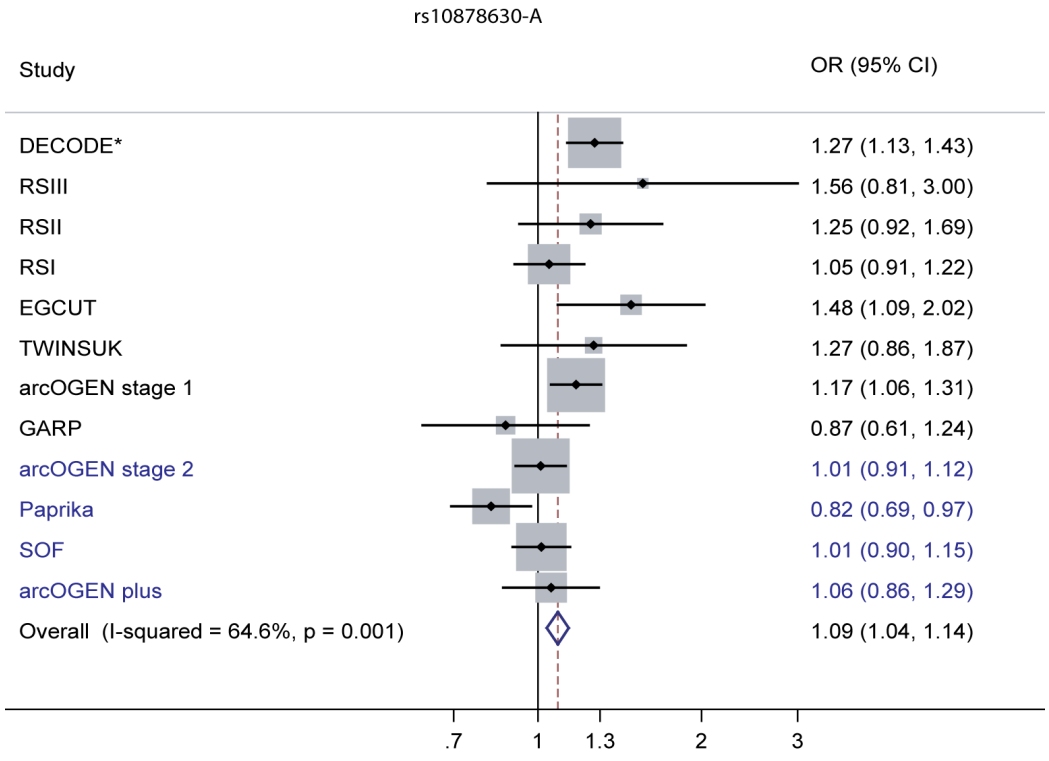


Figure S10

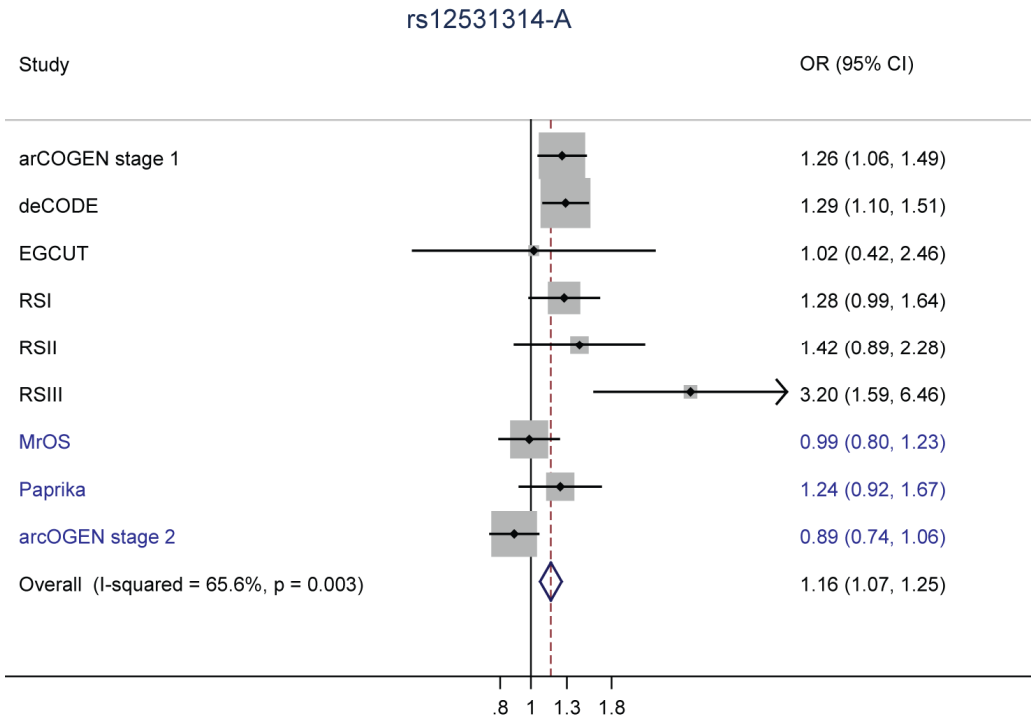
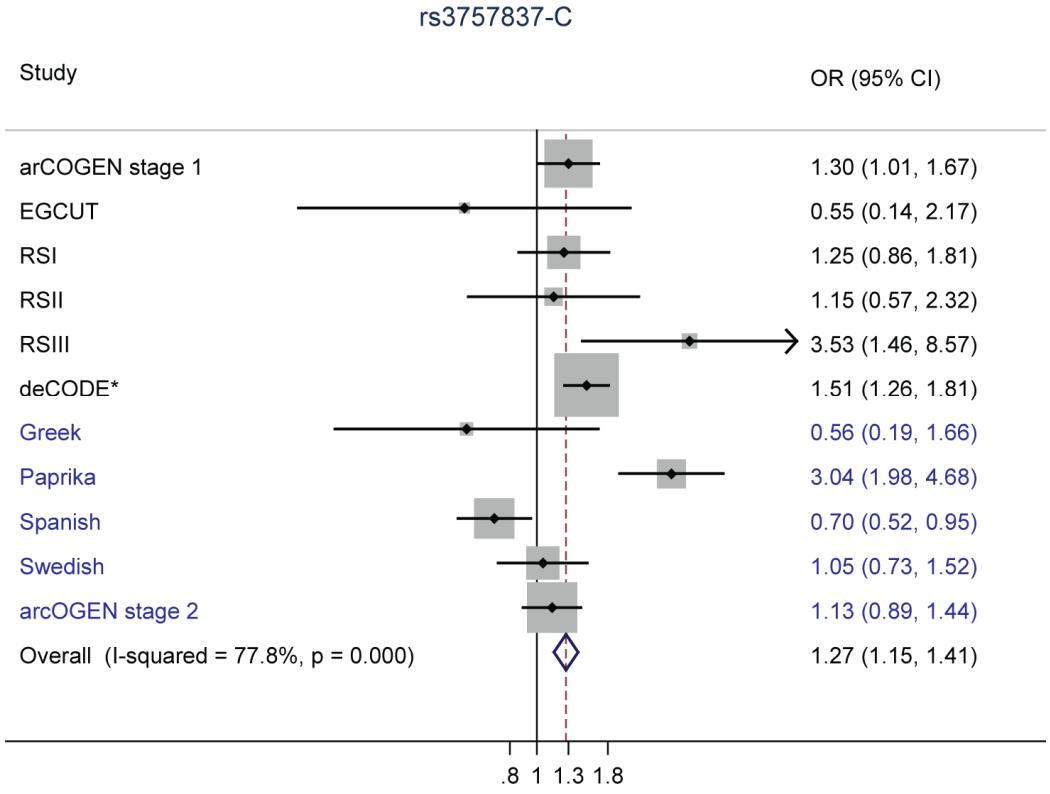


Figure S11



3. Supplementary Tables

Table S1. Summary statistics for cases and controls in the groups that were included in the discovery stage.

Study	N cases	Females (%)	Age Mean (SD)	BMI Mean (SD)	Height Mean (SD)	N controls	Females (%)	Age Mean (SD)	BMI Mean (SD)	Height Mean (SD)
Discovery										
arcOGEN	1728	64	65.8 (8.7)	28.1 (5.4)	165 (9.0)	4896	49.0	NA	NA	NA
deCODE	1423	55	69.7 (7.7)	26.8 (4.5)	169 (9.0)	31385	55	51.3 (21.7)	27.1 (5.3)	170 (9.0)
EGCUT	64	74	71.7 (13.2)	29.5 (4.6)	164.8 (9.2)	2531	56	47.4 (2.2)	25.7 (5.7)	164.3 (6.9)
GARP	106	82	60.1 (7.6)	26.8 (5.4)	168.0 (7.8)	1671	55	57.7 (1.4)	26.2 (5.5)	169.9 (9.3)
RSI	760	53	67.4 (7.7)	26.0 (3.5)	168.0 (9.3)	3233	51	66.9 (7.6)	25.8 (3.4)	168.3 (9.3)
RSII	159	52	64.0 (7.5)	27.0 (4.0)	169.1 (9.3)	1472	51	63.4 (6.9)	26.9 (4.0)	169.3 (9.3)
RSIII	41	56	55.7 (5.4)	27.3 (4.3)	171.2 (9.3)	1487	56	55.6 (5.4)	27.3 (4.3)	171.1 (9.4)
TwinsUK	68	100	56.2 (7.8)	26.0 (4.6)	161.0 (6.3)	228	100	49.0 (5.9)	24.3 (4.0)	162.4 (5.8)

Table S2. Association p-values of the prioritized SNPs before and after adjustments for age, BMI and height

SNP/Group	P Unadjusted	P Age-Adjusted	P BMI-Adjusted	P Height-Adjusted
rs6094710				
arcOGEN	0.0003398	NA	NA	NA
deCODE	0.013	0.028	0.029	0.026
EGCUT	0.226229	0.448463	0.236926	0.379193
GARP	0.42634	0.665	0.407	0.461
RSI	0.01931	0.0142	0.0157	0.01757
RSII	0.03662	0.04299	0.03607	0.02804
RSIII	0.6377	0.49	0.6325	0.499
TWINSUK	0.81883	0.9473	0.5693	0.5272
rs1577792				
arcOGEN	0.0026362	NA	NA	NA
deCODE	0.014	0.017	0.017	0.02
EGCUT	0.592781	0.437926	0.52881	0.526826
GARP	0.18416	0.474	0.223	0.254
RSI	0.001447	0.014	0.001551	0.001623
RSII	0.03931	0.07495	0.03963	0.03916
RSIII	0.8852	0.858	0.8509	0.7973
TWINSUK	0.36496	0.9349	0.332	0.2965
rs5009270				
arcOGEN	0.01702	NA	NA	NA
deCODE	0.0024	0.0023	0.015	0.014
EGCUT	0.0323308	0.0276716	0.0386288	0.0366348
GARP	0.027003	0.041	0.062	0.053
RSI	0.004403	0.002941	0.004449	0.004
RSII	0.4895	0.508	0.479	0.4734
RSIII	0.1292	0.2191	0.1215	0.3647
TWINSUK	0.63171	0.7448	0.8074	0.8516
rs10773046				
arcOGEN	0.0015225	NA	NA	NA
deCODE	0.0000194	0.0000125	0.00029	0.00036
EGCUT	0.756363	0.989485	0.77615	0.831619
GARP	0.47104	0.274	0.367	0.404
RSI	0.6551	0.5028	0.6319	0.6769
RSII	0.1994	0.3065	0.1791	0.1978
RSIII	0.4235	0.1293	0.4174	0.1927
TWINSUK	0.30965	0.4033	0.3059	0.1895
rs17610181				
arcOGEN	0.0034746	NA	NA	NA
deCODE	0.57	0.92	0.3	0.33
EGCUT	NA	0.0549912	0.146748	0.168055
GARP	0.084506	0.085	0.089	0.103
RSI	0.000087	0.0001067	0.000216	0.1851
RSII	0.03551	0.03559	0.03916	0.0302
RSIII	0.5777	0.6116	0.1849	0.1398
TWINSUK	0.91205	0.6116	0.7059	0.6352
rs10878630				

arcOGEN	0.0282	NA	NA	NA
deCODE	0.00014	0.00029	0.0000429	0.0000365
EGCUT	0.0125402	0.0722638	0.0460731	0.0821358
GARP	0.4427	0.182	0.431	0.451
RSI	0.5189	0.5768	0.4333	0.5768
RSII	0.1499	0.1465	0.1564	0.1465
RSIII	0.1882	0.1864	0.1849	0.1854
TWINSUK	0.23472	0.4759	0.2289	0.1637
rs12551314				
arcOGEN	0.0096	NA	NA	NA
deCODE	0.0028	0.0026	0.00044	0.00089
EGCUT	0.962784	0.562756	0.539139	0.659855
GARP	NA	0.705	0.271	0.259
RSI	0.06476	0.05551	0.248	0.06039
RSII	0.1507	0.1674	0.135	0.1449
RSIII	0.001947	0.0009252	0.001961	0.001217
TWINSUK	NA	NA	NA	NA
rs3757837				
arcOGEN	0.03802	NA	NA	NA
deCODE	0.0000122	0.0000234	0.0000243	0.0000381
EGCUT	0.38955	0.203123	0.104614	0.562756
GARP	NA	0.197	0.256	0.296
RSI	0.2497	0.1901	0.2607	0.242
RSII	0.7047	0.8979	0.7049	0.7067
RSIII	0.01069	0.00433	0.005202	0.005141
TWINSUK	NA	NA	NA	NA

Table S3. Association p-values of the prioritized SNPs in publicly available databases of height and BMI

		Height		BMI	
Marker	Locus	P	# individuals	P	# individuals
rs6094710	20q13	0.092	131389	0.96	123206
rs1577792	6q14	0.66	133766	0.046	123861
rs5009270	7q31	0.27	127727	0.63	119547
rs10773046	12q24	2.1E-04	133828	0.42	123863
rs17610181	17q23	0.70	132978	0.13	123864
rs10878630	12q15	0.55	133647	1.00	123718
rs12551314	9q22	0.30	133762	0.65	123866
rs3757837	7p13	0.46	116897	0.81	116638

Acknowledgements

The authors are very grateful to all study participants, the staff from all studies and the participating physicians and pharmacists. TreatOA is funded by the European Commission framework 7 program (grant 200800). The authors thank all arcOGEN participants for their contribution to this manuscript. arcOGEN (http://www.arcogen.org.uk/) was funded by a special purpose grant from Arthritis Research UK (grant 18030). This study used genotype data from population controls that was generated by the Wellcome Trust Case Control Consortium 2 (http://www.wtccc.org.uk), funded by The Wellcome Trust (grant 083948). The population controls were from the 1958 British Birth Cohort collection funded by the Medical Research Council (grant G0000934) and The Wellcome Trust (grant 068545) and from the UK Blood Services Collection of Common Controls funded by The Wellcome Trust. This research utilizes resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. The UK samples used in arcOGEN derive from nine centres; Nottingham, London, Oxford, Sheffield, Southampton, Newcastle, Wansbeck, Worcester and Edinburgh. For Nottingham we acknowledge Arthritis Research UK for funding the collection of the majority of extant cases (stage 1). For London we thank the staff from the TwinsUK unit and the Chingford Study for stage 1 patient ascertainment, we acknowledge financial support from Arthritis Research UK, from the Wellcome Trust, from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre (BRC) award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London, and from the European Commission framework 7 programme TREAT-OA (grant 200800). For Oxford we acknowledge funding support from the Collisson Foundation, the Botnar Foundation and the Jean Shanks Foundation for stage 1 patient ascertainment and we acknowledge the NIHR for supporting the Biomedical Research Unit (BRU) at the University of Oxford. For Sheffield we acknowledge the NIHR for supporting the Sheffield Musculoskeletal BRU collection site, the South Yorkshire Clinical Research Network for part- funding the Sheffield research nurses and for clerical support, the Royal College of Surgeons of England and the Cavendish Hip Foundation. For Southampton we acknowledge the Wellcome Trust Clinical Research Facility at Southampton General Hospital and we thank Anna Bara, trial manager for the Arthritis Research UK-funded VIDEO study. For Edinburgh we acknowledge the contribution of Gayle Scott and Helen Watters for recruitment and ascertainment of stage 2 cases. For Newcastle we acknowledge the Freeman Hospital arthroplasty surgeons Nigel Brewster, David Deehan, Craig Gerrand, Andy Gray, Munawar Hashmi, James Holland and David Weir for assistance in stage 2 patient ascertainment, we thank Karen Bettinson for her assistance, we acknowledge the support of the UK NIHR BRC for Ageing and Age-related disease award to the Newcastle upon Tyne Hospitals NHS Foundation Trust and the support of the Northumberland, Tyne and Wear CLRN. For Wansbeck we acknowledge the Northumbria arthroplastyconsulants Mr Partington, Mr Muller, Mr Emmerson, Mr Prasad, Mr Kramer, Mr Jones, Mr Murty, Mr Asaad, Mr Leitch, Mr Pratt, Mr Townshend and Miss Van Kampen for accrual, and the research nurse Donna Gray for patient ascertainment. For Worcester we acknowledge the support of the West Midlands (south) CLRN. We acknowledge stage 1 and 2 sample management undertaken by the UK DNA Banking Network funded by the Medical Research Council at CIGMR - the Centre for Integrated Genomic Medical Research, University of Manchester. Genotyping was performed at the Wellcome Trust Sanger Institute and we thank Emma Gray, Sarah Edkins and Cordelia Langford for their assistance. We thank Vanessa Cox of the MRC Epidemiology Resource Centre, Southampton, for database and computing services for the arcOGEN stage 2 patient questionnaires. The Study of Osteoporotic Fractures (SOF) is supported by National Institutes of Health funding. The National Institute on Aging (NIA) provides support under the following grant numbers: AG05407, AR35582, AG05394, AR35584, AR35583, R01 AG005407, R01 AG027576-22, 2 R01 AG005394-22A1, R01 AR052000-01 A1, 2K24-AR04884, AR043052 and 2 R01 AG027574-22A1. The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, R01 AR052000-01 A1, 2K24-AR04884, AR043052 and UL1 RR024140. The Leiden University Medical Centre, the Dutch Arthritis Association and Pfizer Inc., Groton, CT, USA support the GARP study, whilst genotypic work was supported by the Netherlands Organization of Scientific Research (MW 904-61-095, 911-03-016, 917 66344 and 911-03-012), Leiden University Medical Centre, and by the “Centre of Medical System Biology” and the “Netherlands Consortium of Healthy Aging” in the framework of the Netherlands Genomics Initiative (NGI). Furthermore, the research leading to these results has received funding from the Dutch Arthritis Association (DAA 2010_017) and the European Union's Seventh Framework Programme (FP7/2007-2011) under grant agreement n° 259679.TwinsUK acknowledges financial support from the Wellcome Trust, the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and Arthritis Research Campaign. The deCODE Genetics OA project

is funded in part by the European Commission framework 7 programme TREAT-OA (grant 200800). The Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) (project nr. 050-060-810) and the Erasmus Medical Center and Erasmus University, Rotterdam. EGCUT received financing by FP7 grants (201413, 245536), grant from Estonian Government SF0180142s08, from the EU through the European Regional Development Fund, OPENGENE, in the frame of Centre of Excellence in Genomics and Univ. of Tartu grant SP1GVARENG. We acknowledge EGCUT and Estonian Biocentre personnel, especially Mr V. Soo. The Study of Osteoporotic Fractures (SOF) is supported by National Institutes of Health funding. The National Institute on Aging (NIA) provides support under the following grant numbers: R01 AG005407, R01 AR35582, R01 AR35583, R01 AR35584, R01 AG005394, R01 AG027574, and R01 AG027576. The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, and UL1 RR024140. The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) provides funding for the MrOS ancillary study 'Replication of candidate gene associations and bone strength phenotype in MrOS' under the grant number R01-AR051124. The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) provides funding for the MrOS ancillary study 'GWAS in MrOS and SOF' under the grant number RC2ARO58973. Spanish and Greek sample genotyping was done with funding from grant 11/01048 and by RETICS Program, RD08/0075 (RIER) of the Instituto de Salud Carlos III (Spain) that are partially financed by the European Regional Development Fund of the European Union. AGD-W, LS, KP and EZ are supported by the Wellcome Trust (grant 098051)

References

1. Marchini J, Howie B, Myers S, McVean G, Donnelly P (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 39: 906-913.
2. Kavvoura FK, Ioannidis JP (2008) Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. Hum Genet 123: 1-14.
3. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557-560.
4. Han B, Eskin E (2011) Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet 88: 586-598.
5. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, et al. (2007) The Rotterdam Study: objectives and design update. Eur J Epidemiol 22: 819-829.
6. Stefansson SE, Jonsson H, Ingvarsson T, Manolescu I, Jonsson HH, et al. (2003) Genomewide scan for hand osteoarthritis: a novel mutation in matrilin-3. Am J Hum Genet 72: 1448-1459.
7. Spector TD, Williams FM (2006) The UK Adult Twin Registry (TwinsUK). Twin Res Hum Genet 9: 899-906.
8. Riyazi N, Meulenbelt I, Kroon HM, Ronday KH, Hellio le Graverand MP, et al. (2005) Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 64: 438-443.
9. Heijmans BT, Beekman M, Houwing-Duistermaat JJ, Cobain MR, Powell J, et al. (2006) Lipoprotein particle profiles mark familial and sporadic human longevity. PLoS Med 3: e495.
10. Panoutsopoulou K, Southam L, Elliott KS, Wrayner N, Zhai G, et al. (2011) Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. Ann Rheum Dis 70: 864-867.
11. arcOGEN Consortium and arcOGEN Collaborators (2012) Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. Lancet 380: 815-823.
12. Fytili P, Giannatou E, Papanikolaou V, Stripeli F, Karachalios T, et al. (2005) Association of repeat polymorphisms in the estrogen receptors alpha, beta, and androgen receptor genes with knee osteoarthritis. Clin Genet 68: 268-277.

13. Rodriguez-Lopez J, Pombo-Suarez M, Liz M, Gomez-Reino JJ, Gonzalez A (2006) Lack of association of a variable number of aspartic acid residues in the asporin gene with osteoarthritis susceptibility: case-control studies in Spanish Caucasians. Arthritis Res Ther 8: R55.
14. Blank JB, Cawthon PM, Carrion-Petersen ML, Harper L, Johnson JP, et al. (2005) Overview of recruitment for the osteoporotic fractures in men study (MrOS). Contemp Clin Trials 26: 557-568.
15. Orwoll E, Blank JB, Barrett-Connor E, Cauley J, Cummings S, et al. (2005) Design and baseline characteristics of the osteoporotic fractures in men (MrOS) study--a large observational study of the determinants of fracture in older men. Contemp Clin Trials 26: 569-585.
16. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, et al. (1995) Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med 332: 767-773.
17. Keurentjes JC, Blane D, Bartley M, Keurentjes JJ, Fiocco M, et al. (2013) Socio-economic position has no effect on improvement in health-related quality of life and patient satisfaction in total hip and knee replacement: a cohort study. PLoS One 8: e56785.
18. Keurentjes JC, Fiocco M, So-Osman C, Onstenk R, Koopman-Van Gemert AWMM et al. (2013) Patients with severe radiographic osteoarthritis have a better prognosis in psysical functioning after hip and knee replacement; A cohort-study. Plos One 8: e59500
19. Keurentjes JC, Fiocco M, Nelissen RG (2013) Willingness to undergo surgery again validated clinically important differences in health-related quality of life after total hip replacement or total knee replacement surgery. J Clin Epidemiol. pii: S0895-4356(13)00171-6. doi: 10.1016/j.jclinepi.2013.04.010.
20. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, et al. (2008) Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 371: 1505-1512.
21. Ju-Hyun P, Wacholder S, Gail MH, Peters U, Jacobs KB et al. (2010) Estimation of effect size distributions from genome-wide association studies and implications for furture discoveries. Nat Genet 42:570-575