Supplementary: Genome-wide association study meta-analysis of chronic widespread pain: evidence for involvement of the 5p15.2 region

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Supplementary Methods:

Full description of the Stage 1 GWAS cohorts.

The ERF study. The ERF study (http://www.epib.nl/research/erf/erf_index.html) is a family-based cohort study that is embedded in the Genetic Research in Isolated Populations Program in the southwest of the Netherlands. The aim of this program was to identify genetic risk factors in the development of complex disorders. For the ERF study, 22 families that had at least five children baptized in the community church between 1850 and 1900 were identified with the help of genealogical records. All living descendants of these couples and their spouses were invited to take part in the study. Data collection started in June 2002 and was finished in February 2005. In this study, we focused on 2,347 participants for whom complete phenotypic, genotypic, and genealogical information was available. The medical ethics committee of Erasmus Medical Center Rotterdam approved the study, and informed consent was obtained from all participants. All participants completed a pain homunculus to report the painful sites in the body (pain during at least half of the days during the last six weeks). Individuals were categorised as CWP cases when they report joint pain in the left side of the body, in the right side of the body, above waist, below waist, and in the axial skeleton. Subjects not being a CWP case were categorised as controls, but subjects using pain medication were excluded from the control group.

The Rotterdam Study. The Rotterdam Study (www.epib.nl/rotterdamstudy) is a prospective, population based cohort study in the district of Rotterdam, the Netherlands. The initial design of the study is straight-forward: a prospective cohort study among, 7,983 persons living in the well-defined Ommoord district in the city of Rotterdam (78% of 10,215 invitees), called Rotterdam Study I (or RS-I). They were all 55 years of age or over and the oldest participant at the start was 106 years. The study started in the second half of 1989. In 1999, 3,011 participants (out of 4,472 invitees) who had become 55 years of age or moved into the study district since the start of the study were added to the cohort, called Rotterdam Study II (or RS-II). In 2006, a further

extension of the cohort was initiated in which 3,932 subjects were included, aged 45–54 years (out of 6,057 invited), called Rotterdam Study III (RS-III). The participants were all examined in some detail at baseline. They were interviewed at home and then had an extensive set of examinations in a specially built research facility in the centre of their district. These examinations were repeated every 3–4 years in characteristics that could change over time. The participants in the Rotterdam Study are followed for a variety of diseases that are frequent in the elderly. Informed consent was obtained from each participant, and the medical ethics committee of the Erasmus Medical Center Rotterdam approved the study. In Rotterdam, the participants completed the same pain homunculus as the subjects of ERF. Individuals were categorised as CWP cases when they report joint pain in the left side of the body, in the right side of the body, above waist, below waist, and in the axial skeleton. Subjects not being a CWP case were categorised as controls, but subjects using pain medication were excluded from the control group.

The TwinsUK study. The TwinsUK cohort (www.twinsuk.ac.uk) is a British adult twin registry shown to be representative of singleton populations and the United Kingdom population. 5687 females aged between the ages of 16-88 completed questionnaires related to chronic widespread pain between 2002 - 2008. These questionnaires asked the participants about any pain in muscles, bones or joints lasting at least one week in the past three months.

Full description of the Replication Cohorts

1958BC. The National Child Development Study, also known as the 1958 British Birth Cohort Study is a large, on-going, prospective cohort study of all children born in England, Scotland, and Wales during one week of March 1958. Detailed methods have been reported previously (Power et al, 2006). Approximately 17,000 participants were recruited at birth and have subsequently been followed up at ages 7, 11, 16, 23, 33, 42 and 45 years. At age 45 years a biomedical survey collected information on health-related factors including the presence of pain.

The sample for the current study was 8,572 individuals who responded to a self-complete pain questionnaire at 45yrs (pain was not joint specific), sent in advance of a nurse interview, and who provided blood samples for genetic analysis.

The AGES Study. Age Gene/Environment Susceptibility Reykjavik (AGES-Reykjavik) Study. The Reykjavik Study cohort originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967. A total of 19,381 people attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow up and was examined in all stages. One group was designated a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5764 survivors of the original cohort who had participated before in the Reykjavik Study. Participants came in a fasting state to the clinic and answered questionnaires related to chronic widespread pain. Informed consent was obtained from all participants. Subjects were asked whether they had pain lasting at least one month in the past 12 months. Questions were asked specifically for hand and wrist, hip, knee, shoulder, feet, toes, ankles and back. The AGES Reykjavik Study GWAS was approved by the intra-mural research program of the National Institute on Aging, by the Iceland National Bioethics Committee (VSN: 00-063) and Data Protection Authority.

The Chingford Study.

The Chingford Study was established in 1989 when 1003 women, aged 44-67 years, derived from the register of a large general practice in Chingford, North London, were recruited to a prospective population-based longitudinal study of osteoarthritis and osteoporosis. In this study, data on joint and spinal pain, collected as part of the year 4 follow-up visit, was used. Subjects were asked whether they had pain in hand, knee, hip, feet and back during the last year. When they had pain, they were asked for how many days the pain lasted during the last month.

The DSDBAC Study. In 1983**,** 6542 healthy individuals aged between 42 and 92 years old resident in Newcastle and Greater Manchester were recruited into a longitudinal population-based study of cognition in healthy old age. Pain manikin data was collected via postal questionnaire on subjects remaining in the cohort in 2007, and additionally, subjects were asked whether they have had pain for more than 3 months. Pain was not asked joint specific.

The EPIFUND Study. EPIFUND is a prospective population-based study of functional disorders. Participants, aged 25-65 years old, were recruited from three primary care registers in the North-west of England. Pain manikin data was collected via a postal questionnaire at baseline and at two follow ups. Additionally to the manikins, subjects were asked whether they have had pain for more than 3 months. Pain was not asked joint specific. DNA was collected using buccal swab sampling from 1189 subjects who participated in all three phases.

The FOA Study. The Framingham Osteoarthritis Study is a population-based multigenerational cohort study of over 3500 participants, and is a sub-study of the larger Framingham Heart Study (FHS). In this study, we focused on study participants with information on widespread pain (collected in FOA) and genetic data (collected in FHS). All participants completed a pain homunculus to report the sites in the body having pain, aching, or stiffness on most days. Individuals were categorised as CWP cases when they report joint pain in the left side of the body, in the right side of the body, above waist, below waist, and in the axial skeleton. Subjects not being a CWP case were categorised as controls, but subjects using pain medication were excluded from the control group.

The GARP Study. The GARP study from Leiden, the Netherlands, consists 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[20]. Written informed consent was obtained from each subject as approved by the ethical committees of the Leiden University Medical Center. We recorded pain in the GARP questionnaire by asking the question: Have you

had pain in and around your joints lasting most days of the last month? Patients could choose: hands (left and/ or right), hips (left and/ or right), knees (left and/ or right), back (cervical, thoracic or lumbar region), shoulders (left and/or right) and other sites as specified. When patients indicated that they had pain in the hands, they could specify the locations in the hand in a drawing. When a patient indicated pain in two sections of two contralateral limbs and in the axial skeleton the patient is defined as a case of CWP. For controls, we used 925 randomly chosen Rotterdam Study participants.

The Hertfordshire Cohort Study (HCS) is a cohort study of men and women born in Hertfordshire, UK during 1931-39 and still living there in adult life. Approximately 3000 participants were recruited in the late 1990s and have subsequently been followed by clinic visit (East Herts only) and postal clinical outcomes questionnaire (all). The sample for the current study was drawn from individuals who completed a pain questionnaire at using a mannequin to report site of pain, and who had previously provided blood samples for genetic analysis. Individuals were categorised having CWP if they reported having pain for at least three months in a detailed pain questionnaire which corresponded pain in the left and right sides of the body, pain both above and below the waist and back pain (pain was not joint specific). Individuals who did not report such pain but reported use of analgesics were excluded from the analysis. All other individuals were categorized as controls resulting in 90 cases and 2117 controls.

The SHIP Study. The SHIP cohort (http://www.medizin.uni-greifswald.de/cm/fv/ship.html) is a prospective, population based cohort study among 4,308 subjects aged ≥20 years from the West Pomerania, Germany. The study was designed to assess prevalence and incidence of risk factors, subclinical disorders and clinical diseases and to investigate associations among them using extensive medical assessments. In this study, we focused on participants for whom complete phenotypic, genotypic, and genealogical information was available. Informed consent was obtained from each participant, and the medical ethics

committee of University of Greifswald approved the study. Subjects were asked to complete questionnaires related to joint pain. These questionnaires asked about pain during the last week, regarding the back, elbow, foot, arms, hands, hip, knee, neck, shoulder, head and facial pain. We decided to exclude head and facial pain, not being joint-related pain. Because no duration was asked for, the pain prevalence in SHIP is one of the highest among the included cohorts.

Genotyping, Quality Control and Imputation

The following sample quality control (QC) criteria were applied in the GWAS of RS-I, RS-II, RS-III and ERF: sample call rate >97.5%, gender mismatch with typed X-linked markers, evidence for DNA contamination in the samples using the mean of the autosomal heterozygosity >0.33, exclusion of duplicates or first-degree relatives estimated by pairwise IBD, exclusion of ethnic outliers (>4 SD from population mean using multidimensional scaling (MDS) analysis with 4 principal components (PCs)), and exclusion of samples with missing pain data, age and/or BMI. In the GWAS of TwinsUK, normalised intensity data was pooled, and genotypes were called on the basis of the Illuminus algorithm[1]. 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. SNPs that had a low call rate (≤90%), Hardy-Weinberg p-values$<10^{-6}$ and minor allele frequencies < 1% were excluded. Samples with call rates <95% were removed.

Genotype imputation was used to evaluate the association of one and the same SNP across samples typed on different genotyping platforms. Genotypes were imputed for all polymorphic SNPs (minor allele frequency >0.01) using either MACH[2] or IMPUTE[3] software, based upon phased autosomal chromosomes of the HapMap CEU Phase II panel (release 22, build 36), orientated on the positive strand. Imputation QC metrics from MACH and IMPUTE were used for filtering out SNPs with low-quality data.

Stage 1 GWAS Meta-Analysis

The estimated inflation factors were 1.176, 1.014, 1.008, 1.006, and 0.989 for ERF, RS-I, RS-II, RS-III, and TwinsUK respectively. SNPs with a minor allele frequency <0.05, a MACH r^2-hat <0.30, or a SNPTEST proper_info <0.40 were excluded from the meta-analysis. We obtained the combined results of the 2,224,068 autosomal SNPs, pooling the effect sizes by means of a fixed effects inverse variance meta-analysis as implemented in METAL. Estimated heterogeneity variance and forest plots were generated using the Comprehensive Meta-Analysis[4] software. Regional association plots of the meta-analysis results were obtained with LocusZoom[5].

Sequenom iPLEX and Taqman Allelic Discrimination genotyping

Genotypes for CHINGFORD, EPIFUND, and HCS were generated using Sequenom iPLEX genotyping and Taqman Allelic Discrimination genotyping. Genomic DNA was extracted from samples of peripheral venous blood according to standard procedures. 1-2 ng genomic DNA was dispensed into 384-wells plates using a Caliper Sciclone ALH3000 pipetting robot (Caliper LS, Mountain View, CA, USA).

For Sequenom iPLEX genotyping, multiplex PCR assays were designed using Assay Designer on the website (https://mysequenom.com/tools/genotyping/default.aspx). For this, sequences containing the SNP site and at least 100 bp of flanking sequence on either side of the SNP were used. Briefly, 2 ng genomic DNA was amplified in a 5 ul reaction containing 1 × Taq PCR buffer (Sequenom), 2 mM $MgCl_2$, 500 uM each dNTP, 100 nM each PCR primer, 0.5 U Taq (Sequenom). The reaction was incubated at 94°C for 4 minutes followed by 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds, 72°C for 1 minute, followed by 3 minutes at 72°C. Excess dNTPs were then removed from the reaction by incubation with 0.3 U shrimp alkaline phosphatase (Sequenom) at 37°C for 40 minutes followed by 5 minutes at 85°C to deactivate the enzyme. Single primer extension over the SNP was carried out in a final concentration of between 0.731

uM and 1.462 uM for each extension primer (depending on the mass of the probe), iPLEX termination mix (Sequenom), 10x iPLEX Buffer Plus and iPLEX enzyme (Sequenom) and cycled using the following program; 94°C for 30 seconds followed by 94°C for 5 seconds, 5 cycles of 52°C for 5 seconds, and 80°C for 5 seconds, the last three steps were repeated 40 times, then 72°C for 3 minutes. The reaction was then desalted by addition of 6 mg clear resin (Sequenom) followed by mixing (15 minutes) and centrifugation (5 min, 3,000rpm) to settle the contents of the tube. The extension product was then spotted onto a 384 well spectroCHIP using the SEQUENOM MassARRAY Nanodispenser RS1000 before analysis on the MassARRAY Compact System (Sequenom). Data collection was performed using SpectroACQUIRE 3.3.1.13 and clustering was called using TYPER Analyzer 4.0.3.18 (Sequenom). Additionally to ensure data quality genotypes for each subject were also checked manually.

For Taqman Allelic Discrimination genotyping (Applied Biosystems Inc., Foster City, CA, USA), all SNP assays were available at www.appliedbiosystems.com as pre-designed assays. The PCR reaction mixture included 1-2 ng of genomic DNA in a 2 µl volume and the following reagents: FAM and VIC probes (200 nM), primers (0.9 uM), 2x Taqman PCR master mix (Applied Biosystems Inc., Foster City, CA, USA). Reagents were dispensed in a 384-well plate using the Deerac Equator NS808 (Deerac Fluidics, Dublin, Ireland). PCR cycling reaction were performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, CA, USA) and consisted of initial denaturation for 15 minutes at 95° C, and 40 cycles with denaturation of 15 seconds at 95° C and annealing and extension for 60 seconds at 60° C. Results were analysed by the ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc., Foster City, CA, USA).

RNA isolation and real-time PCR for mRNA quantitation

Total RNA was isolated with the Trizol (Invitrogen, Paisley, UK) method and 1 µg of total RNA was used to synthesize cDNA with SuperScript Reverse Transcriptase (Invitrogen;

carrageenan experiment) or iScript^{TM} Select cDNA Synthesis Kit (Invitrogen; CFA experiment) using random hexamers.

Using quantitative PCR, mRNA levels of Cct5 and Fam173b were measured in the spinal cord and the DRG. The real-time PCR reaction with SYBR green Master mix (Bio-Rad, Alphen aan den Rijn, the Netherlands) was performed on the iQ5 Real-Time PCR Detection System (BioRad; carrageenan experiment) or Mastercycler ep realplex (Eppendorf; CFA experiment). For both experiments, the gene expression levels were normalized for Gapdh and β-actin expression levels (housekeeping genes). We used the following primers:

- Cct5 forward: GTCTCATGGGGCTTGAGG, reverse: GTCCGCATTGTGTTTGCTAC.
- Fam173b forward: TGGTGTGCCCCAGATGAT, reverse: TGCCCTCTCCAGTGGTGT.
- Gapdh forward: TGAAGCAGGCATCTGAGGG, reverse: CGAAGGTGGAAGAGTGGGAG.
- β-actin forward: AGAGGGAAATCGTGCGTGAC, reverse: CAATAGTGATGACCTGGCCGT.

We designed the primers to be intron-spanning thereby targeting the first two exons of Cct5 and the last two exons of Fam173b. The thermocycling profile of amplification was 10 min at 95°C, 40 cycles of 15s at 95°C and 1 min at 60°C, 1 min at 95°C, and 2 min at 65°C, followed by a final meltcurve analysis.

Supplementary Figures and Tables:

Figure S1A. Heat withdrawal latency time measurements at day 0 and day 6 after intraplantar carrageenan (n=4) or saline (n=4) injection. The latency time was measured using the Hargreaves Test. Data are expressed as means ± SEM. *** = $p<0.001$.

Figure S1B. Heat withdrawal latency time measurements at day 0, day 1, and day 3 after intraplantar Complete Freund's Adjuvant (CFA) (n=4) or saline (n=4) injection. The latency time was measured using the Hargreaves Test. Data are expressed as means ± SEM. *** = $p<0.001$.

Table S1. Pain assessment methods according to the different studies.

Study	Method	Areas of the body checked									Duration of pain*
		Back	*Elbow*	*Foot*	*Hand*	*Hip*	*Knee*	*Neck*	*Shoulder*	*Others*	
Stage 1											
ERF study	Homunculus (drawing circles)	yes	yes	yes	yes	yes	yes	yes	yes	no	> half of the days during last 6 weeks
RS-I	Homunculus (drawing circles)	yes	yes	yes	yes	yes	yes	yes	yes	no	> half of the days during last 6 weeks
RS-II	Homunculus (drawing circles)	yes	yes	yes	yes	yes	yes	yes	yes	no	> half of the days during last 6 weeks
RS-III	Homunculus (drawing circles)	yes	yes	yes	yes	yes	yes	yes	yes	no	> half of the days during last 6 weeks
TWINSUK	Questionnaire	yes ±	no ±	yes ±	yes ±	no ±	no ±	yes ±	yes ±	arm, leg, chest±	> 1 week during the last 3 months
Stage 2a											
1958BC	Homunculus (shading areas)	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	>=3 months
AGES	Questionnaire	yes	no	yes, (not s.s.)º	yes,(not s.s.)º	yes	yes	no	yes	no	> 1 month during last year
DSDBAC	Homunculus (shading areas)	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	>=3 months
FOA	Homunculus (shading areas)	yes	yes	yes (incl. indiv. joints)#	yes (incl. indiv. joints)#	yes	yes	yes	yes	ankle, wrist	"pain on most days"
GARP	Questionnaire	yes	yes	no	yes	yes	yes	yes	no	spine	not asked
SHIP	Questionnaire	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	head, face, arm, abdomen, pelvic ±	not asked
Stage 2b											
CHINGFORD	Joint Symptom Questionnaire	yes	no	no	yes	yes	yes	yes	no	no	not asked
EPIFUND	Homunculus (shading areas)	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	yes ±	>=3 months
HCS	Homunculus (shading areas)	yes ±	yes ±	yes ±	yes ±	no ±	yes ±	yes ±	no ±	upper arm, lower arm, upper leg, lower leg, sternum, chest, abdomen, buttock ±	"pain lasting most days of the month over the last year" – extra question: pain lasted > 3 months?

* If available, the duration of pain criteria was used; # Incl. indiv. joints: the individual joints were scored; º Not s.s.: information about this joint was not side specific. ± Not joint specific.

ERF study = Erasmus Rucphen Family study; RS = Rotterdam Study; TwinsUK = The UK Adult Twin Registry; 1958BC = 1958 Birth Cohort; AGES = Age, Gene/Environment Susceptibility study Reykjavik; CHINGFORD = Chingford 1000 Women Study; DSDBAC = Dyne Steel DNA Bank for Ageing and Cognition; EPIFUND = EPIdemiological study of FUNctional Disorders study; FOA = Framingham Osteoarthritis Study; GARP = Genetics OsteoArthritis and Progression study Leiden; HCS = Hertfordshire Cohort Study. SHIP = Study of Health In Pomerania.

Table S2. Study design, number of cases and controls, and sample quality control for the Stage 1 GWAS cohorts.

Study		Study design	Total sample size (N)	Sample QC		Number of samples in the analyses	References
Short name	**Full name**			**Call rate**	**Other exclusions**		
ERF study	Erasmus Rucphen Family study	Family Based Cohort	2300	> 95%	1) Excess heterozygosity based on FDR 2) Ethnic outliers 3) Gender mismatch 4) Missing phenotype	149 cases, 665 controls	[6]
RS-I	Rotterdam Study I	Population Based Cohort	7983	≥ 97.5%	1) Gender mismatch with typed Xlinked markers; 2) Excess autosomal heterozygosity > 0.336~FDR>0.1%; 3) Duplicates and/or 1st or 2nd degree relatives using IBS probabilities > 97% from PLINK; 4) Ethnic outliers using IBS distances > 3SD from PLINK; 5) Missing pain, age, and BMI information.	563 cases, 1892 controls	[7]
RS-II	Rotterdam Study II	Population Based Cohort	3011	≥ 97.5%	1) Gender mismatch with typed X-linked markers; 2) Excess autosomal heterozygosity (F<-0.055); 3) Duplicates and/or 1st degree relatives using IBD piHAT >40% from PLINK; 4) Ethnic outliers using IBS distances > 4SD mean HapMap CEU cluster from PLINK; 5) Missing pain, age, and BMI information.	110 cases, 668 controls	[7]
RS-III	Rotterdam Study III	Population Based Cohort	3932	≥ 97.5%	1) Gender mismatch with typed Xlinked markers; 2) Excess autosomal heterozygosity (F<-0.055); 3) Duplicates and/or 1st degree relatives using IBD piHAT >40% from PLINK; 4) Ethnic outliers IBS distances > 4SD mean HapMap CEU cluster from PLINK; 5) Missing pain, age, and BMI information.	85 cases, 868 controls	[7]
TwinsUK	UK Adult Twin Registry	Twins Based Cohort	5687	≥ 95%	1) Heterozygosity <33% or >67%; 2) Ethnic outliers; 3) Related individuals and duplicates; 4) Missing pain, age, and BMI information.	401 cases, 1698 controls	[8, 9]

ERF study = Erasmus Rucphen Family study; RS = Rotterdam Study; TwinsUK = The UK Adult Twin Registry.

Table S3. Information on genotyping methods, quality control of SNPs, and imputation for the Stage 1 GWAS cohorts.

Cohort	Genotyping						Imputation		
	Platform	Genotype Calling Algorithm	Inclusion Criteria			SNPs that met QC criteria	Imputation Software	Inclusion Criteria	
			MAF	Callrate	p-value for HWE			MAF	Imputation Quality Score
ERF study	Illumina 318K, 370K, Affymetrix 250K	BRLMM, BeadStudio	>0.5%	>95%	$>10^{-06}$	NA	MACH	>0%	r^2-hat ≥ 0.30
RS-I	Illumina / HumanHap 550K V.3, Illumina / HumanHap 550K V.3 DUO;	BeadStudio Genecall	≥1%	≥97.5%	$>10^{-06}$	512,349	MACH	≥0%	(O/E)σ2 ratio≥0.1 r^2-hat ≥ 0.30
RS-II	Illumina / HumanHap 550 V.3 DUO; Illumina / HumanHap 610 QUAD	Genomestudio Genecall	≥1%	≥97.5%	$>10^{-06}$	466,389	MACH	≥1%	(O/E)σ2 ratio≥0.1 r^2-hat ≥ 0.30
RS-III	Illumina / HumanHap 610 QU	Genomestudio Genecall	≥1%	≥97.5%	$>10^{-06}$	514,073	MACH	≥1%	(O/E)σ2 ratio≥0.1 r^2-hat ≥ 0.30
TwinsUK	Illumina / HumanHap 300 & 550	Illuminus	≥1%	≥95.0%	$>10^{-06}$	295,702	IMPUTE	>0%	proper-info ≥ 0.40

ERF study = Erasmus Rucphen Family study; RS = Rotterdam Study; TwinsUK = The UK Adult Twin Registry.

Table S4. Study design, number of cases and controls, and sample quality control for the Stage 2 cohorts.

Study		Study design	Total sample size (N)	Sample QC		Number of samples in the analyses	References
Short name	Full name			Call Rate	Other exclusions		
1958BC	National Child Development (1958 Birth Cohort) Study	Prospective Birth Cohort	4958	≥97%	1) Autosomal heterozygosity 2) Non-Caucasian 3) Average difference in probe intensities across SNPs 4) Individuals with >5% IBD 5) Gender mismatch 6) >10% discordance upon repeated genotyping 7) Missing phenotype	315 cases, 2206 controls	[10, 11]
AGES	Age, Gene Environment Susceptibility Reykjavik Study	Population Based Cohort	3219	≥97%	1) Sample failure 2) Genotype mismatch with reference panel 3) Sex mismatch	173 cases, 1204 controls	[12]
DSDBAC	Dyne Steel DNA Bank for Ageing and Cognition	Population Based Cohort	6542	>95%	1) Gender mismatch 2) IBD sharing >0.25 3) Non-Caucasians by multi-dimensional scaling	81 cases, 219 controls	[13]
FOA	The Framingham Osteoarthritis Study	Population Based Cohort	4792	≥97%	1) Subject heterozygosity > ± 5 SDs from the the mean 2) Missing pain, age, and BMI information	384 cases, 814 controls	[14]
GARP	Genetics osteoARthritis and Progression Study	Case Based Cohort	384	>99%	NA	67 cases, 925 RS controls	[15]
SHIP	Study of Health In Pomerania	Population Based Cohort	4081	>92%	1) Duplicate samples (by IBS) 2) Reported/genotyped gender mismatches	183 cases, 589 controls	[16, 17]
CHINGFORD	Chingford Study	Population Based Cohort	831	NA	1) Missing phenotype information.	48 cases, 337 controls	[18, 19]
EPIFUND	EPIdemiological study of FUNctional Disorders	Population Based Cohort	6290	>95%	None	139 cases, 503 controls	[20]
HCS	Hertfordshire Cohort Study	Population Based Cohort	1073	>95%	Missing phenotype information.	90 cases, 2117 controls	[21]

1958BC = 1958 Birth Cohort; AGES = Age, Gene/Environment Susceptibility study Reykjavik; CHINGFORD = Chingford 1000 Women Study; DSDBAC = Dyne Steel DNA Bank for Ageing and Cognition; EPIFUND = EPIdemiological study of FUNctional Disorders study; FOA = Framingham Osteoarthritis Study; GARP = Genetics OsteoArthritis and Progression study Leiden; HCS = Hertfordshire Cohort Study. SHIP = Study of Health In Pomerania.

Table S5. Information on genotyping methods, quality control of SNPs, and imputation for the Stage 2 cohorts.

Cohort	Genotyping						Imputation		
	Platform	Genotype Calling Algorithm	Inclusion Criteria			SNPs that met our replication QC criteria	Imputation Software	Inclusion Criteria	
			MAF	Callrate	p-value for HWE			MAF	Imputation Quality Score
1958BC	Affymetrix v6.0 (WTCCC2), Illumina 1.2M chip (WTCCC2), and Illumina 550k (T1DGC)	Chiamo software (adapted for Affymetrix 6.0 SNP data) and Illuminus	>5%	≥ 98%	>0.05	2 SNPs + 7 proxy SNPs	NA	NA	NA
AGES	Illumina 370 CNV BeadChip	BeadStudio	≥ 1%	≥97%	$>10^{-6}$	10	MACH	>0%	r2-hat ≥ 0.30
DSDBAC	Illumina610-Quadv1 chip	Genomestudio	≥1%	≥98%	$>10^{-3}$	10	MACH	>0%	r^2-hat ≥ 0.30
FOA	Affymetrix 500K and 50K supplemental array	BRLMM	≥1%	≥97%	$\geq 10^{-6}$	10	MACH	≥1%	r2-hat ≥ 0.3
GARP	Illumina Human660W	Genome studio	>5%	>98%	>0.001	10	IMPUTE	>5%	proper-info ≥ 0.85
SHIP	Affymetrix SNP Array 6.0	Birdseed2	≥ 0%	≥ 0%	≤ 1	10	IMPUTE v0.5.0	≥ 0%	proper_info ≤ 1
CHINGFORD	Sequenom iPLEX and Taqman Allelic Discrimination	Taqman / Sequenom	NA	≥95%	>0.05	10	NA	NA	NA
EPIFUND	Sequenom iPLEX	Sequenom	NA	≥95%	>0.05	1 SNP + 1 proxy SNP	NA	NA	NA
HCS	Taqman Allelic Discrimination	Taqman	NA	NA	NA	5	NA	NA	NA

1958BC = 1958 Birth Cohort; AGES = Age, Gene/Environment Susceptibility study Reykjavik; CHINGFORD = Chingford 1000 Women Study; DSDBAC = Dyne Steel DNA Bank for Ageing and Cognition; EPIFUND = EPIdemiological study of FUNctional Disorders study; FOA = Framingham Osteoarthritis Study; GARP = Genetics OsteoArthritis and Progression study Leiden; HCS = Hertfordshire Cohort Study. SHIP = Study of Health In Pomerania.

Table S6. Genotyped SNPs for replication**.**

Study	rs13361160	rs12132674	rs7680363	rs7835968	rs2249104	rs17796312	rs4837492	rs11606304	rs524513	rs8065610
1958BC	P: rs1508850	P: rs2843016	P: rs12511202	P: rs7830100	P: rs3858511	P: rs12609590	G	NG	P: rs3895875	G
AGES	I	I	I	I	I	I	I	I	I	G
CHINGFORD	G	G	G	G	G	G	G	G	G	G
DSDBAC	I	I	I	I	I	I	G	I	I	G
EPIFUND	G	P: rs2843016	NG	NG	NG	NG	NG	NG	NG	NG
FOA	I	I	I	I	I	I	I	I	I	I
GARP	I	I	I	I	I	I	G	I	I	G
HCS	G	G	G	NG	NG	NG	NG	G	NG	G
SHIP	I	I	G	I	I	I	G	I	I	G

G = Genotyped, I = Imputed, NG = Not Genotyped, P = Proxy Genotyped. The rs-number called behind P gives the used proxy.

1958BC = 1958 Birth Cohort; AGES = Age, Gene/Environment Susceptibility study Reykjavik; CHINGFORD = Chingford 1000 Women Study; DSDBAC = Dyne Steel DNA Bank for Ageing and Cognition; EPIFUND = EPIdemiological study of FUNctional Disorders study; FOA = Framingham Osteoarthritis Study; GARP = Genetics OsteoArthritis and Progression study Leiden; HCS = Hertfordshire Cohort Study. SHIP = Study of Health In Pomerania.

Table S7. Mean age and BMI for all participating studies

Cohort	Mean and Standard Deviation of the covariates age and BMI					
	Age (y)			BMI (kg/m²)		
	Cases	**Controls**	**P-value** Cases vs. Controls	**Cases**	**Controls**	**P-value** Cases vs. Controls
ERF study	51.4 (+/- 11.2)	45.3 (+/- 14.3)	1.10E-06	27.1 (+/- 5.1)	26.1 (+/- 4.6)	1.30E-02
RS-I	68.9 (+/- 8.6)	69.5 (+/- 9.0)	1.48E-01	27.4 (+/- 4.3)	26.6 (+/- 4.1)	1.00E-06
RS-II	67.2 (+/- 7.6)	68.0 (+/- 7.5)	2.66E-01	29.0 (+/- 5.0)	27.7 (+/- 4.3)	3.88E-03
RS-III	57.8 (+/- 7.8)	56.2 (+/- 5.8)	1.85E-02	28.6 (+/- 5.2)	27.4 (+/- 5.0)	4.23E-02
TwinsUK	55.4 (+/- 11.6)	51.1 (+/- 13.9)	6.50E-10	26.6 (+/- 5.4)	24.7 (+/- 4.3)	2.70E-11
1958BC	NA	NA	NA	27.7 (+/- 6.1)	26.7 (+/- 5.3)	4.00E-3
AGES	75.7 (+/- 5.2)	76.6 (+/- 5.6)	6.36E-1	28.6 (+/- 4.8)	27.2 (+/- 4.8)	1.44E-5
DSDBAC	79.7 (+/-4.7)	80.3 (+/-5.7)	4.09E-1	NA	NA	NA
FOA	61.0 (+/- 11.3)	58.5 (+/- 13.6)	2.00E-3	28.6 (+/- 5.8)	25.6 (+/- 4.8)	2.00E-16
GARP	58.5 (+/-6.9)	57.7 (+/- 1.4)	7.00E-3	28.1 (+/- 6.1)	26.3 (+/- 5.9)	1.40E-2
SHIP	60.7 (+/- 12.9)	56.7 (+/- 13.4)	1.30E-3	29.3 (+/-5.41)	27.6 (+/- 5.136)	5.00E-5
CHINGFORD	57.2 (+/-5.9)	56.5 (+/-8.7)	5.89E-1	27.1 (+/-4.6)	26.3 (+/-4.5)	3.29E-1
EPIFUND	50.9 (+/-8.8)	48.5 (+/-10.4)	1.37E-2	NA	NA	NA
HCS	66.8 (+/- 2.7)	66.4 (+/-2.8)	4.88E-1	29.7 (+/- 6.1)	26.8 (+/- 4.5)	1.69E-5

ERF study = Erasmus Rucphen Family study; RS = Rotterdam Study; TwinsUK = The UK Adult Twin Registry; 1958BC = 1958 Birth Cohort; AGES = Age, Gene/Environment Susceptibility study Reykjavik; CHINGFORD = Chingford 1000 Women Study; DSDBAC = Dyne Steel DNA Bank for Ageing and Cognition; EPIFUND = EPIdemiological study of FUNctional Disorders study; FOA = Framingham Osteoarthritis Study; GARP = Genetics OsteoArthritis and Progression study Leiden; HCS = Hertfordshire Cohort Study. SHIP = Study of Health In Pomerania.

Table S8. Information on statistical analysis for the Stage 1 GWAS cohorts.

Cohort	Association Analyses		
	SNPs in meta-analysis	**Analysis Software**	**References Analysis Software**
ERF study	2,463,846	ProbABEL	[22]
RS-I	2,542,336	GRIMP (MACH2DAT)	[2, 23]
RS-II	2,536,671	GRIMP (MACH2DAT)	[2, 23]
RS-III	2,533,563	GRIMP (MACH2DAT)	[2, 23]
TwinsUK	2,460,943	PLINK	[24]

ERF study = Erasmus Rucphen Family study; RS = Rotterdam Study; TwinsUK = The UK Adult Twin Registry.

Table S9: 29 proxy SNPs which are in LD with rs13361160 (r^2>0.1).

SNP	Proxy	Distance	r^2	D'	Gene Variant	GeneName	Possible eQTL-effect?
rs13361160	rs1045392	56655	0.216	0.892	3'UTR	FAM173B	
rs13361160	rs1045369	57016	0.216	0.892	3'UTR	FAM173B	
rs13361160	rs17294394	58695	0.105	0.329	Intronic	FAM173B	
rs13361160	rs4557374	59018	0.144	1.000	Intronic	FAM173B	
rs13361160	rs7716217	59785	0.216	0.892	Intronic	FAM173B	
rs13361160	rs7716565	60006	0.135	1.000	Intronic	FAM173B	
rs13361160	rs7716851	60151	0.144	1.000	Intronic	FAM173B	
rs13361160	rs7736719	60169	0.144	1.000	Intronic	FAM173B	
rs13361160	rs6887347	61868	0.215	1.000	Intronic	FAM173B	
rs13361160	rs16884328	62099	0.109	0.534	Intronic	FAM173B	
rs13361160	rs6887590	62124	0.144	1.000	Intronic	FAM173B	
rs13361160	rs6888157	62153	0.144	1.000	Intronic	FAM173B	
rs13361160	rs6880482	64235	0.144	1.000	Intronic	FAM173B	
rs13361160	rs2292264	65667	0.186	1.000	Intronic	FAM173B	
rs13361160	rs7710415	65930	0.135	1.000	Intronic	FAM173B	
rs13361160	rs16884348	66129	0.154	1.000	Intronic	FAM173B	yes: r^2 with eQTL-SNP rs2445871 = 0.872
rs13361160	rs12653481	67213	0.186	1.000	Intronic	FAM173B	
rs13361160	rs2438652	69438	0.165	1.000	Non-synonymous-coding	FAM173B	yes: r^2 with eQTL-SNP rs2445871 = 0.818
rs13361160	rs2445871	70170	0.135	1.000	Intronic	FAM173B	yes: rs2445871 is an eQTL SNP
rs13361160	rs2607326	70968	0.134	0.851	Intronic	FAM173B	
rs13361160	rs2607328	71623	0.125	1.000	Intronic	FAM173B	
rs13361160	rs2607298	82296	0.104	0.639	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.953
rs13361160	rs2445867	83341	0.104	0.639	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.953
rs13361160	rs1042392	86338	0.159	0.854	Synonymous coding	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.904
rs13361160	rs2028274	86787	0.104	0.639	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.953
rs13361160	rs2028272	86899	0.111	0.646	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.909
rs13361160	rs7710938	87070	0.123	0.732	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.951
rs13361160	rs7729006	87146	0.123	0.732	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.951
rs13361160	rs2438653	89550	0.104	0.639	Intronic	CCT5	yes: r^2 with eQTL-SNP rs2244964 = 0.953

Table S10. Association results of 92 candidate SNPs in the stage 1 GWAS meta-analysis sorted by gene names and P-values. The strongest associated SNPs (p<0.01) were shaded (P<0.01).

MarkerName	Gene	Allele1	Allele2	Freq A1	OR	95%CI	P-value
rs2020917	**COMT**	**t**	**c**	**0.266**	**0.856**	**(0.772-0.948)**	**2.97E-03**
rs5993883	**COMT**	**t**	**g**	**0.496**	**1.142**	**(1.046-1.247)**	**3.05E-03**
rs5746846	COMT	c	g	0.501	1.098	(1.005-1.200)	3.85E-02
rs1544325	COMT	a	g	0.457	1.087	(0.995-1.187)	6.38E-02
rs2239393	COMT	a	g	0.603	1.081	(0.986-1.184)	9.51E-02
rs4818	COMT	c	g	0.604	1.081	(0.986-1.184)	9.66E-02
rs4646312	COMT	t	c	0.604	1.078	(0.984-1.181)	1.07E-01
rs165728	COMT	t	c	0.935	0.812	(0.615-1.071)	1.41E-01
rs4680	*COMT*	*a*	*g*	*0.532*	*1.057*	*(0.963-1.162)*	*2.44E-01*
rs4633	COMT	t	c	0.536	1.048	(0.957-1.147)	3.09E-01
rs174699	COMT	t	c	0.929	0.860	(0.632-1.172)	3.41E-01
rs2097903	COMT	a	t	0.500	0.961	(0.875-1.056)	4.08E-01
rs165774	COMT	a	g	0.313	1.034	(0.933-1.146)	5.21E-01
rs5993882	COMT	t	g	0.768	1.027	(0.924-1.143)	6.20E-01
rs174674	COMT	a	g	0.274	1.020	(0.917-1.134)	7.19E-01
rs10483639	**GCH1**	**c**	**g**	**0.203**	**0.846**	**(0.755-0.948)**	**3.84E-03**
rs752688	**GCH1**	**t**	**c**	**0.202**	**0.846**	**(0.755-0.948)**	**3.94E-03**
rs4411417	**GCH1**	**t**	**c**	**0.798**	**1.181**	**(1.055-1.323)**	**4.00E-03**
rs8007267	GCH1	t	c	0.178	0.890	(0.790-1.001)	5.23E-02
rs3783641	GCH1	a	t	0.192	0.902	(0.803-1.013)	8.15E-02
rs599548	**OPRM1**	**a**	**g**	**0.142**	**1.189**	**(1.050-1.346)**	**6.41E-03**
rs558025	OPRM1	a	g	0.739	1.085	(0.979-1.203)	1.19E-01
rs1799971	OPRM1	a	g	0.873	0.907	(0.796-1.033)	1.42E-01
rs563649	OPRM1	t	c	0.091	1.088	(0.934-1.268)	2.78E-01
rs2075572	OPRM1	c	g	0.551	1.051	(0.957-1.154)	3.00E-01
rs10485171	CNR1	a	g	0.530	1.104	(1.009-1.207)	3.09E-02
rs1078602	CNR1	a	g	0.485	0.941	(0.861-1.030)	1.88E-01
rs6454674	CNR1	t	g	0.705	0.957	(0.869-1.055)	3.81E-01
rs2400707	ADRB2	a	g	0.444	0.909	(0.831-0.994)	3.68E-02
rs1042714	ADRB2	c	g	0.548	1.117	(1.000-1.246)	4.90E-02
rs12654778	ADRB2	a	g	0.367	1.072	(0.978-1.175)	1.39E-01
rs1042713	ADRB2	a	g	0.363	1.070	(0.976-1.173)	1.48E-01
rs17778257	ADRB2	a	t	0.630	0.941	(0.859-1.032)	1.96E-01
rs1042718	ADRB2	a	c	0.162	1.027	(0.912-1.156)	6.62E-01

MarkerName (II)	Gene	Allele1	Allele2	Freq A1	OR	95%CI	P-value
rs8192619	TAAR1	a	g	0.056	0.732	(0.517-1.035)	7.77E-02
rs12584920	HTR2A	t	g	0.187	0.903	(0.804-1.015)	8.75E-02
rs4941573	HTR2A	a	g	0.578	0.975	(0.891-1.066)	5.72E-01
rs6313	HTR2A	a	g	0.419	1.019	(0.932-1.114)	6.77E-01
rs17289394	HTR2A	a	g	0.400	0.992	(0.906-1.085)	8.55E-01
rs10502058	GRIA4	t	c	0.884	0.888	(0.774-1.019)	9.18E-02
rs2510177	GRIA4	a	g	0.090	1.140	(0.976-1.331)	9.72E-02
rs10895837	GRIA4	t	c	0.116	1.122	(0.977-1.287)	1.03E-01
rs642544	GRIA4	t	g	0.583	0.960	(0.878-1.051)	3.78E-01
rs17104711	GRIA4	a	g	0.084	1.039	(0.887-1.217)	6.38E-01
rs8065080	TRPV1	t	c	0.618	0.929	(0.848-1.017)	1.09E-01
rs1143623	IL-1B	c	g	0.721	0.923	(0.836-1.019)	1.12E-01
rs16944	IL-1B	a	g	0.344	1.076	(0.980-1.182)	1.25E-01
rs12621220	IL-1B	t	c	0.277	1.080	(0.979-1.192)	1.26E-01
rs1143627	IL-1B	a	g	0.656	0.937	(0.853-1.029)	1.74E-01
rs1143634	IL-1B	a	g	0.244	0.952	(0.856-1.058)	3.61E-01
rs1143643	IL-1B	t	c	0.336	0.987	(0.897-1.086)	7.91E-01
rs1143633	IL-1B	t	c	0.337	0.988	(0.898-1.087)	8.03E-01
rs3917368	IL-1B	t	c	0.335	0.990	(0.901-1.089)	8.42E-01
rs1800469	TGFb	a	g	0.299	0.931	(0.843-1.029)	1.61E-01
rs11661134	MC2R	a	g	0.069	0.859	(0.692-1.067)	1.70E-01
rs11627241	SERPINA6	t	c	0.257	0.931	(0.839-1.033)	1.78E-01
rs746530	SERPINA6	a	g	0.327	0.940	(0.854-1.034)	2.01E-01
rs941601	SERPINA6	t	C	0.135	0.954	(0.837-1.087)	4.76E-01
rs1998056	SERPINA6	c	g	0.413	1.019	(0.931-1.115)	6.86E-01
rs8022616	SERPINA6	a	g	0.892	1.005	(0.867-1.165)	9.48E-01
rs1800871	IL-10	a	g	0.220	0.931	(0.834-1.038)	1.99E-01
rs1800872	IL-10	t	g	0.221	0.931	(0.834-1.039)	2.02E-01
rs1800896	IL-10	t	c	0.492	0.968	(0.886-1.057)	4.73E-01
rs1800890	IL-10	a	t	0.600	1.001	(0.910-1.103)	9.77E-01
rs1875999	CRHBP	t	c	0.683	0.948	(0.863-1.041)	2.61E-01
rs17561	IL-1A	a	c	0.304	0.950	(0.862-1.047)	3.02E-01
rs1800587	IL-1A	a	g	0.304	0.951	(0.862-1.048)	3.08E-01

MarkerName (III)	Gene	Allele1	Allele2	Freq A1	OR	95%CI	P-value
rs3813034	SLC6A4	a	c	0.538	1.044	(0.955-1.141)	3.46E-01
rs11842874	MCF2L	a	g	0.931	1.098	(0.903-1.335)	3.51E-01
rs2239704	TNF / LTA	a	c	0.387	0.957	(0.872-1.050)	3.54E-01
rs6746030	SCN9A	a	g	0.140	1.058	(0.932-1.202)	3.81E-01
rs454078	IL-1RN	a	t	0.730	0.964	(0.874-1.064)	4.72E-01
rs315952	IL-1RN	T	c	0.705	0.990	(0.899-1.090)	8.31E-01
rs1048101	ADRA1A	a	g	0.562	0.971	(0.889-1.061)	5.19E-01
rs1946518	IL-18	t	g	0.397	1.028	(0.940-1.125)	5.38E-01
rs1799945	HFE	c	g	0.858	1.035	(0.910-1.177)	6.01E-01
rs2842003	RGS4	t	g	0.428	1.021	(0.929-1.123)	6.65E-01
rs6280	DRD3	t	c	0.676	1.021	(0.929-1.123)	6.69E-01
rs1020759	NFKb	t	c	0.402	0.980	(0.896-1.073)	6.69E-01
rs4129256	TAAR2	a	g	0.203	1.024	(0.917-1.143)	6.72E-01
rs2745428	TAAR2	a	c	0.788	0.993	(0.892-1.106)	9.03E-01
rs5275	PTGS2	a	g	0.671	1.020	(0.929-1.121)	6.75E-01
rs4906902	GABRB3	a	g	0.827	1.026	(0.910-1.156)	6.80E-01
rs2069827	IL-6	t	g	0.058	0.960	(0.781-1.179)	6.96E-01
rs1800795	IL-6	c	g	0.409	1.004	(0.916-1.099)	9.39E-01
rs1800797	IL-6	a	g	0.399	0.997	(0.909-1.093)	9.44E-01
rs1554606	IL-6	t	g	0.428	0.998	(0.913-1.091)	9.72E-01
rs2069845	IL-6	a	g	0.572	1.001	(0.916-1.095)	9.76E-01
rs7911	GBP1	a	g	0.637	0.994	(0.907-1.090)	9.05E-01
rs6265	BDNF	t	c	0.198	1.006	(0.900-1.124)	9.22E-01

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