Clin Orthop Relat Res (2018) 0:1-13
DOI 10.1097/01.blo.0000533629.49193.09

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

2018 Hip Society Proceedings

The 2018 Otto Aufranc Award: How Does Genome-wide Variation Affect Osteolysis Risk After THA?

Scott J. MacInnes PhD, Konstantinos Hatzikotoulas PhD, Anne Marie Fenstad MSc, Karan Shah PhD, Lorraine Southam PhD, Ioanna Tachmazidou PhD, Geir Hallan PhD, Hårvard Dale PhD, Kalliope Panoutsopoulou PhD, Ove Furnes PhD, Eleftheria Zeggini PhD, J. Mark Wilkinson PhD

Received: 1 December 2017 / Accepted: 4 April 2018 / Published online: 0, XXXX

Abstract

Background Periprosthetic osteolysis resulting in aseptic loosening is a leading cause of THA revision. Individuals vary in their susceptibility to osteolysis and heritable factors may contribute to this variation. However, the overall contribution that such variation makes to osteolysis risk is unknown.

Questions/purposes We conducted two genome-wide association studies to (1) identify genetic risk loci associated with susceptibility to osteolysis; and (2) identify genetic risk loci associated with time to prosthesis revision for osteolysis.

Methods The Norway cohort comprised 2624 patients after THA recruited from the Norwegian Arthroplasty Registry, of whom 779 had undergone revision surgery for osteolysis. The UK cohort included 890 patients previously

This study was funded by Arthritis Research UK grants 21163 and 18030 (EZ, JMW), Western Norway Regional Health Authority (OF), the National Institute for Health Research funded Sheffield Bone Biomedical Research Unit (SJM, JMW), and the Wellcome Trust (grant 098051) (EZ).

These studies were approved by the National Research Ethics Service in England (NRES 12/YH/0390, October 30, 2012) and by the Directorate of Health in Norway (Ref. 08/8916, October 20, 2008) and the regional Ethical Committee of Western Norway (Ref. 08/10018, September 8, 2008). All work was conducted in accordance with the ethical standards in the 1964 Declaration of Helsinki, and all participants provided written informed consent.

This work was performed at the University of Sheffield, Sheffield, UK; Haukeland University Hospital, Bergen, Norway; and the Wellcome Trust Sanger Institute, Cambridge, UK.

S. J. MacInnes, K. Shah, J. M. Wilkinson, Department of Oncology and Metabolism, University of Sheffield, The Medical School, Sheffield, UK

K. Hatzikotoulas, I. Tachmazidou, K. Panoutsopoulou, E. Zeggini, Wellcome Trust Sanger Institute, Cambridge, UK

A. M. Fenstad, H. Dale, The Norwegian Arthroplasty Register, Department of Orthopaedic Surgery, Haukeland University Hospital, Bergen, Norway

L. Southam, Wellcome Trust Centre for Human Genetics, Oxford, UK

G. Hallan, O. Furnes, Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Bergen, Norway

J. M. Wilkinson (✉), Room EU14, Department of Oncology and Metabolism, University of Sheffield, The Medical School, Beech Hill Road, Sheffield, S10 2JF, UK, email: j.m.wilkinson@sheffield.ac.uk

Wolters Kluwer

recruited from hospitals in the north of England, 317 who either had radiographic evidence of and/or had undergone revision surgery for osteolysis. All participants had received a fully cemented or hybrid THA using a small-diameter metal or ceramic-on-conventional polyethylene bearing. Osteolysis susceptibility case-control analyses and quantitative trait analyses for time to prosthesis revision (a proxy measure of the speed of osteolysis onset) in those patients with osteolysis were undertaken in each cohort separately after genome-wide genotyping. Finally, a meta-analysis of the two independent cohort association analysis results was undertaken.

Results Genome-wide association analysis identified four independent suggestive genetic signals for osteolysis case-control status in the Norwegian cohort and 11 in the UK cohort ($p \leq 5 \times 10^{-6}$). After meta-analysis, five independent genetic signals showed a suggestive association with osteolysis case-control status at $p \leq 5 \times 10^{-6}$ with the strongest comprising 18 correlated variants on chromosome 7 (lead signal rs850092, $p = 1.13 \times 10^{-6}$). Genome-wide quantitative trait analysis in cases only showed a total of five and nine independent genetic signals for time to revision at $p \leq 5 \times 10^{-6}$, respectively. After meta-analysis, 11 independent genetic signals showed suggestive evidence of an association with time to revision at $p \leq 5 \times 10^{-6}$ with the largest association block comprising 174 correlated variants in chromosome 15 (lead signal rs10507055, $p = 1.40 \times 10^{-7}$).

Conclusions We explored the heritable biology of osteolysis at the whole genome level and identify several genetic loci that associate with susceptibility to osteolysis or with premature revision surgery. However, further studies are required to determine a causal association between the identified signals and osteolysis and their functional role in the disease.

Clinical Relevance The identification of novel genetic risk loci for osteolysis enables new investigative avenues for clinical biomarker discovery and therapeutic intervention in this disease.

Introduction

Despite improvements in modern prosthetic design, 5% to 10% of THA prostheses undergo revision within 10 years [28, 32]. Although osteolysis after THA has been mitigated by the use of highly crosslinked polyethylene bearings [22], osteolysis and its sequelae aseptic loosening remain a leading indication for revision surgery, accounting for 55% of THA revision procedures worldwide [43]. Revision surgery carries a three- to eightfold greater in-hospital mortality, higher morbidity, and poorer functional outcome versus primary THA [9, 31, 58].

Aseptic loosening is the clinical endpoint of periprosthetic osteolysis, which describes a progressive resorption of bone caused by a host inflammatory response to particulate wear debris [15, 25, 44, 45]. This inflammatory bone loss is mediated by proinflammatory cytokines that upregulate osteoclastogenesis directly or indirectly through receptor activator of nuclear factor κB ligand signaling [7, 19-21, 24, 52, 56] while also downregulating osteoblastogenesis [27]. The exact mechanisms involved in this process are still not fully understood, although several studies have implicated innate immune signaling through pattern recognition receptor activation by danger and pathogen-associated molecular patterns [3, 5, 41, 48].

Evidence from in vitro studies suggest that individuals vary in their immunologic response to wear debris [12, 37]; however, the component of osteolysis that is attributable to heritable factors remains unclear. Similarly, the genes that modulate the time after surgery when osteolysis occurs in patients who develop the disease also remain relatively unexplored and may differ from those that modulate susceptibility. Several investigators have explored the relationship between genetic variation within candidate genes and susceptibility to periprosthetic osteolysis with the first identified association being with the promoter region of the gene encoding tumor necrosis factor (TNF) [53]. Subsequently, several associations between single nucleotide polymorphism (SNPs) in proinflammatory cytokines and bone turnover pathways and osteolysis have been identified [1, 2, 11, 13, 14, 26, 30, 33-35, 47, 51]. However, our knowledge of the genetics of osteolysis is currently based entirely on studies using the "candidate" gene approach in which the threshold for identifying an association is low. The only genetic association with osteolysis identified to date that has been independently replicated is found at the TNF promoter [11, 53].

Candidate gene studies, which are based on a priori hypotheses about the role of a selected gene or a group of pathway-related genes, have several limitations. These include low sample sizes leading to low statistical power to detect modest to small effect sizes that are characteristic of most complex diseases and incomplete coverage of variation across the genes of interest. Limited knowledge of the etiopathogenesis of disease also restricts the selection of candidate genes and misses variation in genes lying in previously unsuspected pathways. In contrast, genome-wide association studies utilize a hypothesis-free approach enabling the examination of a set of maximally informative markers capturing variation across the whole genome. This approach has established thousands of reproducible associations with complex diseases (https://www.ebi.ac.uk/gwas/) [23, 40]. To date, there have been no systematic studies of the genetic architecture of osteolysis at the whole genome level.

We conducted two genome-wide association studies and a subsequent meta-analysis to (1) identify genetic risk

loci associated with susceptibility to osteolysis; and (2) identify genetic risk loci associated with time to prosthesis revision for osteolysis.

Patients and Methods

The Norwegian cohort comprised patients with osteolysis and osteolysis-free matched control patients after THA. The participants were identified from the Norwegian Arthroplasty Register and recruited by postal return of a saliva sample for DNA extraction between April 2009 and December 2011. All patients had previously undergone primary cemented or hybrid (cemented femur) THA for idiopathic osteoarthritis. The recruitment strategy for the Norwegian cohort was planned to minimize confounders between the patients with osteolysis and those in the control group as follows: All live patients recorded in the Norwegian Arthroplasty Register as having had a revision for the indication of osteolysis or aseptic loosening (n = 2029) were invited to participate. The revision patients were recruited first and the control group patients individually matched at a ratio of approximately three to one to be of the same age (± 2 years), sex, implant fixation method, bearing couple material and head size (22-mm or 28-mm bearing only), and year of primary surgery (± 2 years). Patients who had undergone primary THA for an inflammatory arthropathy, femoral neck fracture, secondary osteoarthritis, or who had a history of infection were excluded. Patients who had previously undergone revision arthroplasty were also excluded as were those of self-reported non-European Caucasian ancestry. This exclusion criteria were also confirmed at genotype screening. In all, 923 patients who had previously undergone revision surgery for osteolysis responded to the invitation and provided a saliva sample for DNA analysis. A matched group of 1957 patients identified within the Norwegian Arthroplasty Register as having primary THA for idiopathic osteoarthritis and with no recorded revision surgery episodes for the operated hip provided a saliva sample as disease-negative controls.

The 890 patients in the UK cohort had been previously recruited into a research program examining the genetics of osteolysis, having previously undergone either cemented or hybrid THA with a metal-on-conventional polyethylene bearing couple for primary osteoarthritis. The osteolysis group comprised 317 patients with any osteolysis, with or without aseptic loosening, diagnosed on plain AP and lateral radiographs of the hip using the Harris criteria [16, 17], and the control group comprised 573 asymptomatic patients at a minimum of 7 years after primary THA and who had not undergone any revision surgery and were free from plain radiographic evidence of osteolysis at the time of recruitment. These participants were identified through hospital records from the north of England and recruited between April 2000 and August 2010 as part of previous ethically approved osteolysis studies [13, 30, 53] and had DNA archived in the South Yorkshire and North Derbyshire Musculoskeletal Biobank.

In both cohorts, patients in the osteolysis group were younger, and a greater proportion were men when compared with the control group (Table 1). Patients in the control population also had a longer time since primary THA than the patients with osteolysis, and a greater proportion in the Norway cohort had fully cemented prostheses and ceramic-on-polyethylene bearing couples. These findings are consistent with known osteolysis risk factors [15, 18, 49] and were adjusted for by inclusion as covariates in all subsequent analyses.

DNA Sample Quality Control, Genotyping, and Association Analyses

Genomic DNA from the Norwegian cohort was genotyped on the Infinium Illumina HumanCoreExome-24 BeadChip Kit (Illumina, San Diego, CA, USA). Genotypes were called using the Illumina Genome Studio Gencall calling algorithm. All samples underwent standard quality control (QC) procedures with exclusion criteria as follows: (1) call rate < 80%; (2) gender discrepancy; (3) excess heterozygosity (separately for minor allele frequency (MAF) ≥ 1% and < 1%); (4) duplicates and/or related; (5) ethnicity outliers; and (6) Fluidigm concordance (this identity check looks at sample concordance between Illumina and Fluidigm genotypes). Variants were excluded based on the following: (1) call rate < 98%; (2) Hardy-Weinberg Equilibrium (HWE) $p \leq 1 \times 10^{-4}$; (3) cluster separation score < 0.4; (4) MAF < 0.01; and (5) less than four minor allele counts in cases and controls separately. In total, after the exclusion of samples and variants that failed the QC criteria, 779 osteolysis patients, 1845 control patients, and 508,957 directly typed SNPs remained. Phasing and imputation were carried out remotely on the Haplotype Reference Consortium (HRC) free servers using IMPUTE2 and SHAPEIT3 software (http://www.haplotype-reference-consortium.org/). Briefly, the HRC reference panel consists of 64,976 human haplotypes at 39,235,157 SNPs using whole-genome sequence data from 20 studies of predominantly European ancestry [38]. After imputation and additional QC exclusions (variants with MAF < 0.05, HWE $p \leq 10^{-4}$, and imputation info score ≤ 0.4), the number of variants reached 5,397,933 and 5,397,567 for case-control status and time-to-revision analyses, respectively. In all, 2624 individuals (779 patients with osteolysis and 1845 patients in the control group) passed the QC criteria and were used in the case-control analysis, and in the time-to-revision analysis, only cases were used.

Table 1. Patient characteristics for Norwegian and UK cohorts after genotyping quality control

Norwegian cohort			
Characteristics	**Control group (n = 1845)**	**Osteolysis group (n = 779)**	**p value**
Age at THA (years)	66 ± 7	64 ± 7	< 0.0001
Sex (men/women)	734/1111	348/431	0.01
Fixation (cemented/hybrid)	1737/108	721/58	0.135
Bearing couple (MoP/CoP)	1534/311	701/78	< 0.0001
Time since index THA (years)	14 ± 5	9 ± 5	< 0 .0001
UK cohort			
Characteristics	**Control group (n = 573)**	**Osteolysis group (n = 317)**	**p value**
Age at THA (years)	66 ± 9	61 ± 9	< 0.0001
Sex (men/women)	225/348	174/143	< 0.0001
Fixation (cemented/hybrid)	466/107	291/26	< 0.0001
Bearing couple (MoP/CoP)	573/0	573/0	
Time since index THA (years)	11 ± 6	10 ± 5	0.005

Values are mean ± SD; analyses are t-test or chi square test; MoP = metal-on-polyethylene; CoP = ceramic-on-polyethylene.

Genomic DNA from patients in the UK cohort was genotyped using the Illumina 610k beadchip. After QC, the data set was phased and imputed using the HRC reference panel by applying the same QC metrics used for the Norwegian cohort. After QC, 5,314,896 variants in 890 individuals proceeded to case-control analysis and 5,415,184 variants in 317 individuals proceeded to time-to-revision analysis.

Association analyses for osteolysis case-control status and time to revision in those patients with osteolysis were conducted separately for the Norwegian and UK cohorts and made using the frequentist likelihood ratio test and method ml in SNPTEST v2.5.2 (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/) [36]. To account for population stratification, the first 10 principal components were included as covariates in the association testing. Sex, age at operation, prosthesis fixation method, bearing couple material combination, and lysis-free survival were also used as covariates in the association analysis. The same covariates were used for the time-to-prosthesis revision analysis. Because of the large number of variants tested in genomic studies and the variable levels of linkage (nonindependence) between the variables, p values of $\leq 5 \times 10^{-6}$ were taken as indicating a suggestive association between variant and disease status and $p \leq 5 \times 10^{-8}$ as indicating genome-wide significance. Power was calculated using Quanto v1.2.4 [38] using $p = 5 \times 10^{-8}$ and fixed the sample size to the size of each cohort separately.

Meta-analysis

We performed a meta-analysis of the two analyzed cohorts using the fixed-effects inverse-variance weighted model implemented in METAL (http://www.sph.umich.edu/csg/abecasis/metal/) [54]. The total sample size in the combined cohort consisted of 1096 patients and 2418 control group participants. Variants with per-cohort MAF 0.05, imputation information score > 0.4, and HWE $p \geq 10^{-4}$ were included in the analysis. To test the heterogeneity of the results, we computed Cochran's Q and the I^2 statistic.

Data Availability

Anonymized genotypes of the Norwegian cohort included in this study are publicly available through the European Genome-Phenome Archive (EGA) under accession number EGAS00001001883, data set EGAD00010001289.

Results

Genetic Loci Associated With Osteolysis Case-control Status

In the Norwegian cohort, we found a total of 12 SNPs comprising four independent signals (Manhattan plot [Fig. 1A], QQ plot [Fig. 1B]) that were associated with osteolysis case-control status at $p \leq 5 \times 10^{-6}$. A summary of the loci associated with osteolysis is shown (see Table, Supplemental Digital Content 1). The variant with the most statistically significant p value was rs8101944, an upstream variant of PLPP2 (phospholipid phosphatase 2; PPAP2C [phosphatidic acid phosphatase type 2C]) on chromosome 19 (effect allele [EA] T, effect allele frequency [EAF] 0.06, odds ratio [OR], 0.68; 95% confidence interval [CI], 0.51-0.89; $p = 1.26 \times 10^{-6}$).

In the UK cohort, we identified a total of 61 SNPs comprising 11 independent signals (Manhattan plot [Fig. 2A],

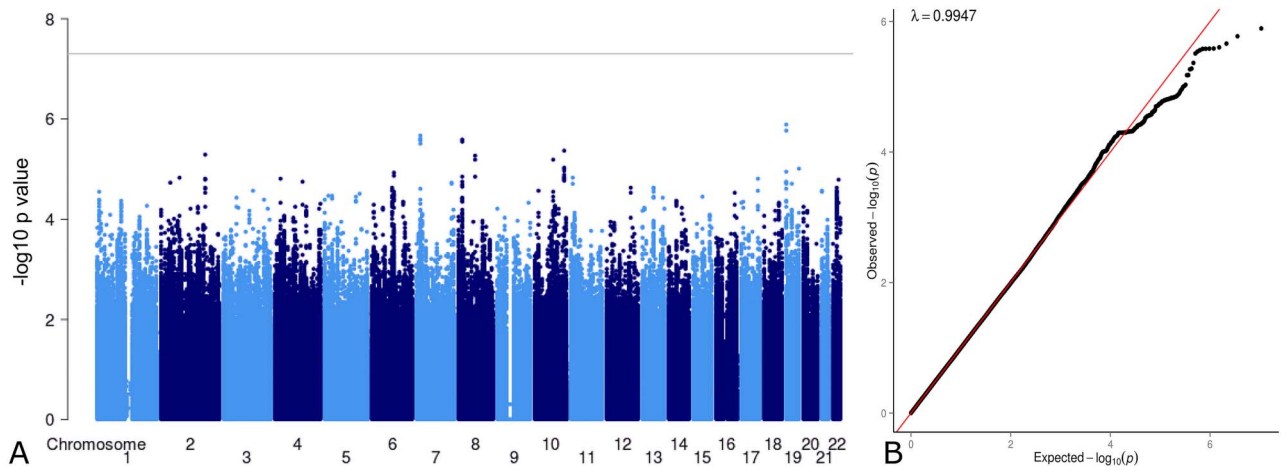

Fig. 1 A-B (**A**) Manhattan plot of the Norwegian cohort case-control status analysis showing the -log10 p values for each variant (y axis) plotted against their respective chromosomal position (x axis) and illustrating four independent genetic association peaks in 779 patients with osteolysis versus 1845 osteolysis-free patients. (**B**) Graph showing QQ plot of the p values for the Norwegian cohort case-control status, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

QQ plot [Fig. 2B]) that were associated with osteolysis case-control status at $p \leq 5 \times 10^{-6}$. A summary of the loci associated with osteolysis case-control status is shown (see Table, Supplemental Digital Content 2). The variant with the most statistically significant p value was rs12135813, an intergenic variant (EA C, EAF 0.37; OR, 0.60; 95% CI, 0.49–0.74; $p = 4.34 \times 10^{-7}$) and lies between the PLXNA2 (plexin A2) and MIR205HG genes on chromosome 1.

Genetic Loci Associated With Time to Prosthesis Revision

In the Norwegian cohort, we identified 32 SNPs comprising five independent signals (Manhattan plot [Fig. 3A], QQ plot [Fig. 3B]) that were associated with time to revision at $p \leq 5 \times 10^{-6}$. A summary of the loci associated with time to revision is shown (see Table, Supplemental Digital Content 3).

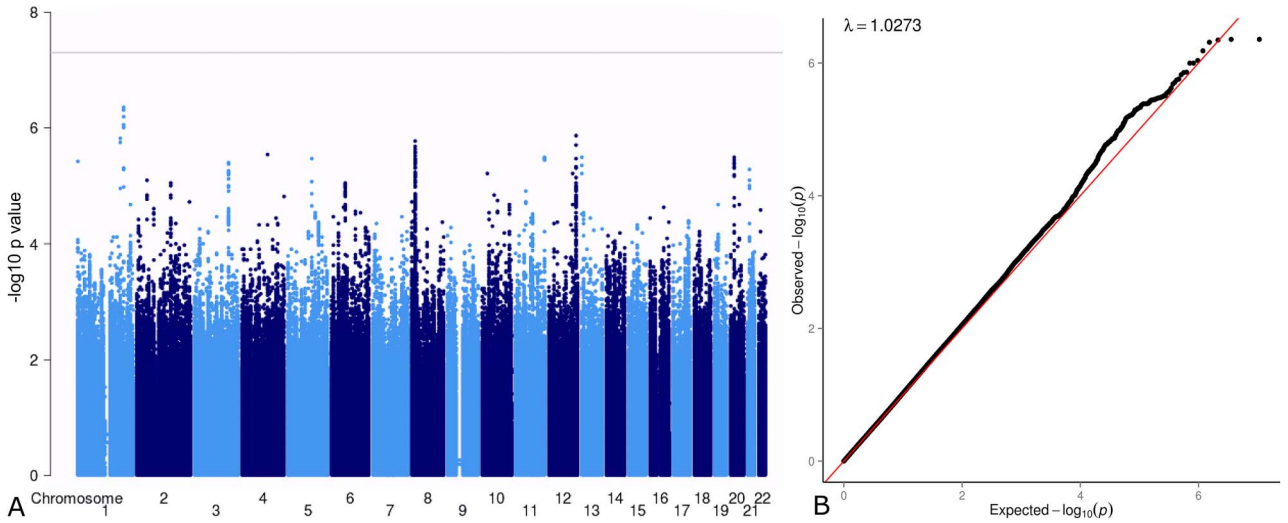

Fig. 2 A-B (**A**) Manhattan plot of the UK cohort case-control status analysis showing the -log10 p values for each variant (y axis) plotted against their respective chromosomal position (x axis) and illustrating 11 independent genetic association peaks in 317 patients with osteolysis versus 573 osteolysis-free patients. (**B**) Graph showing QQ plot of the p values for the UK cohort case-control status association analysis, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

The variant with the most statistically significant p value is rs282329 (EA T, EAF 0.66; beta ± standard error [SE] 0.25 ± 0.05; p = 3.06 x 10^{-7}) and lies between the VEZT (vezatin, adherens junctions transmembrane protein) and METAP2 (methionyl aminopeptidase 2) protein coding genes on chromosome 12.

Genome-wide analysis in the UK cohort identified 19 signals comprising nine independent signals (Manhattan plot [Fig. 4A], QQ plot [Fig. 4B]) that were associated with time to revision at p ≤ 5 x 10^{-6}. A summary of the loci associated with time-to-revision status is shown (see Table, Supplemental Digital Content 4). The variant with the most statistically significant p value was rs184396151 (EA G, EAF 0.67; beta ± SE 1.34 ± 0.17; p = 6.70 x 10^{-7}) and lies within CUX2 (cut-like homeobox 2) protein coding gene on chromosome 12.

Genetic Loci Association Meta-analyses

The results showed that 5,411,522 variants with MAF ≥ 0.05 were common to both the Norwegian and UK osteolysis case-control analyses. After meta-analysis, no signals approached the genome-wide significance threshold of p ≤ 5 x 10^{-8} (Manhattan plot [Fig. 5A], QQ plot [Fig. 5B]). A summary of the loci that were associated with osteolysis case-control status at p ≤ 5 x 10^{-6} is shown (see Table, Supplemental Digital Content 5). Twenty-nine SNPs, with the same direction of effect in both cohorts and comprising five independent signals, showed suggestive evidence for an association with osteolysis susceptibility with p ≤ 5 x 10^{-6}. The strongest signal was in chromosome 7 (Fig. 6) with 18 correlated variants showing p ≤ 5 x 10^{-6}. The lead variant rs850092 (EA A, EAF 0.72; OR, 1.41; 95% CI, 1.23–1.61; p = 1.13 x 10^{-6}) is located within DPY19L2P3 (DPY19L2 pseudogene 3).

In a meta-analysis across the Norwegian and UK cohorts for time to revision, a total of 5,418,572 variants were analyzed (Manhattan plot [Fig. 7A], QQ plot [Fig. 7B]). A summary of the loci that were associated with time to revision at p ≤ 5 x 10^{-6} is shown (see Table, Supplemental Digital Content 6). In all, 209 variants with the same direction of effect in both cohorts and comprising 11 independent signals showed suggestive evidence for an association with time-to-prosthesis revision with p ≤ 5 x 10^{-6}. rs10507055 (Fig. 8) had the most statistically significant p value (EA T, EAF 0.37; beta ± SE -0.22 ± 0.04; p = 1.40 x 10^{-7}) and is in the same region of chromosome 12 as rs282329, which had the most statistically significant p value in the Norwegian cohort association analysis. A block of 174 correlated variants with p ≤ 5 x 10^{-6} was found in chromosome 15 (Fig. 9). rs12899987 is the lead variant and lies within the gene OTUD7A (EA T, EAF 0.81; beta ± SE 0.26 ± 0.05; p = 2.80 x 10^{-7}).

Discussion

Although osteolysis after THA has been mitigated substantially by the use of highly crosslinked polyethylene

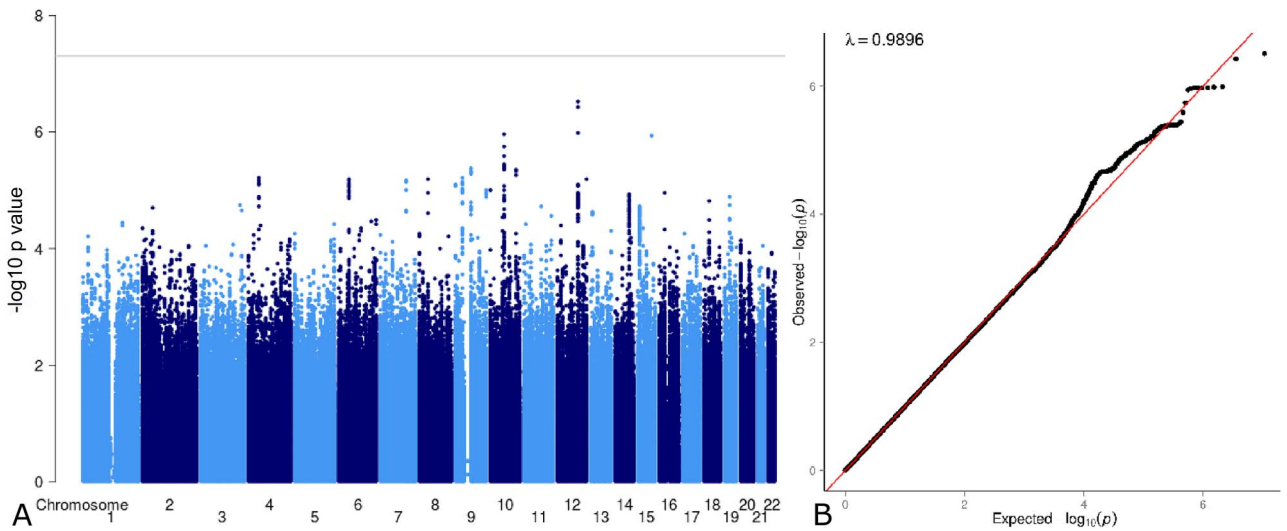

Fig. 3 A-B (**A**) Manhattan plot of the Norwegian cohort time to revision association analysis in osteolysis patients only (n = 779) showing the -log10 p values for each variant (y-axis) plotted against their respective chromosomal position (x-axis) and illustrating five independent genetic association peaks. (**B**) Graph showing QQ plot of the p values for the Norwegian cohort time to revision association analysis, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

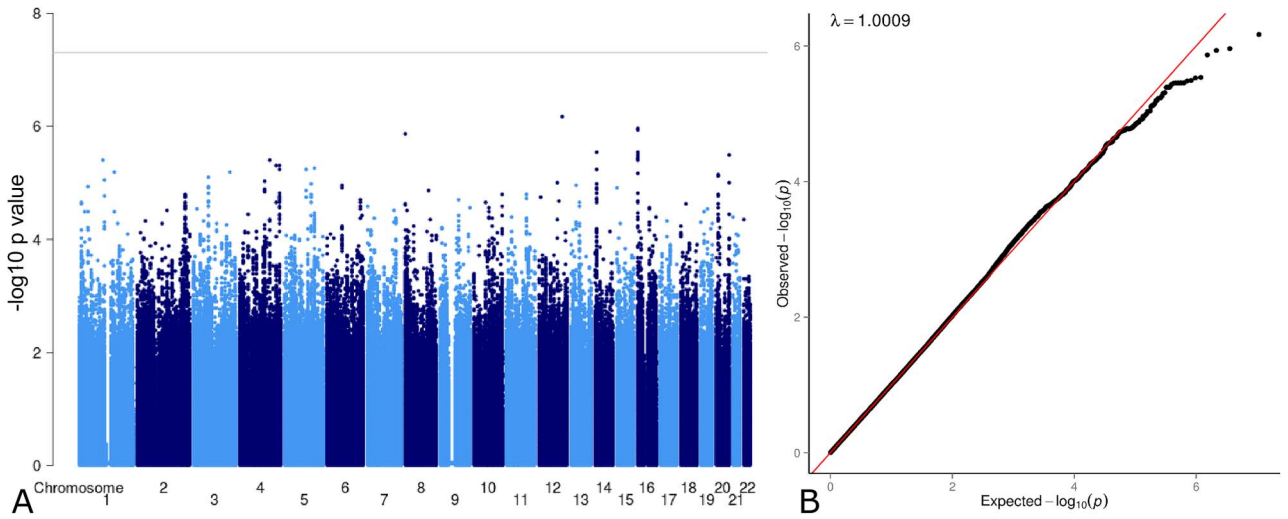

Fig. 4 A-B (**A**) Manhattan plot of the UK cohort time to revision association analysis in osteolysis patients only (n = 317) showing the -log10 p values for each variant (y axis) plotted against their respective chromosomal position (x axis) and illustrating nine independent genetic association peaks. (**B**) Graph showing QQ plot of the p values for the UK cohort time to revision association analysis, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

bearings, osteolysis and its sequelae aseptic loosening remain a leading indication for revision surgery. Previous studies have observed that interindividual differences in susceptibility to osteolysis may have a genetic basis [1, 2, 34, 53], but this question has not been examined systematically at the genome-wide level. In this study, in two European cohorts, we explored the contribution that variation across the human genome makes to osteolysis and found evidence of a modest heritable contribution to disease susceptibility. We found replicating evidence for

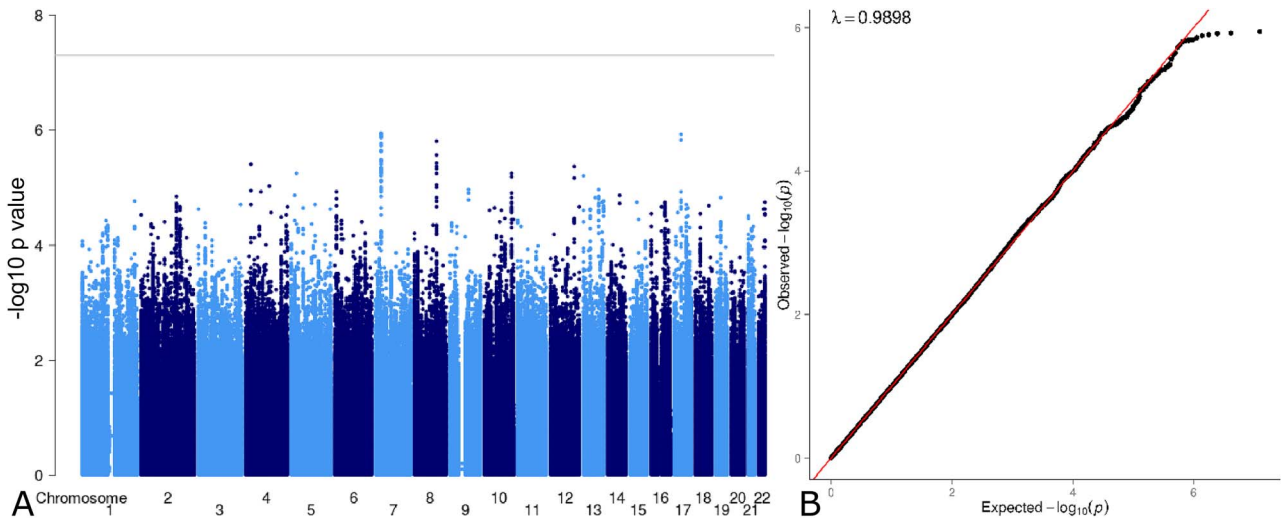

Fig. 5 A-B (**A**) Manhattan plot of the Norwegian and UK cohort case-control status meta-analysis showing the -log10 p values for each variant (y-axis) plotted against their respective chromosomal position (x-axis) and illustrating five independent genetic association peaks in 1096 patients with osteolysis versus 2418 osteolysis-free patients. (**B**) Graph showing QQ plot of the p values for the Norwegian and UK cohort case-control status meta-analysis, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

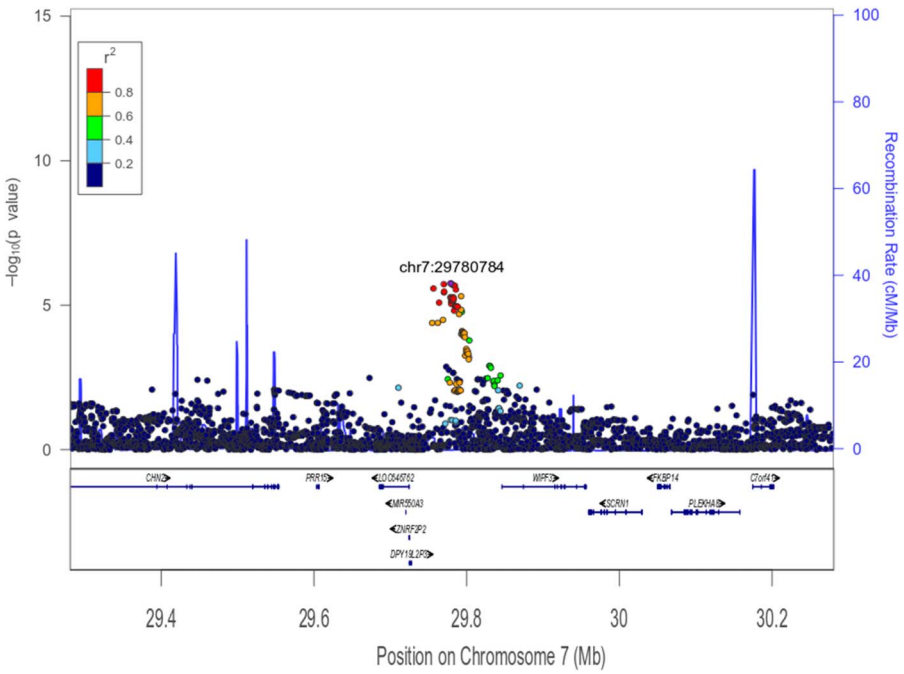

Fig. 6 Regional association plot showing the lead osteolysis susceptibility signal at rs850092 in the case-control association meta-analysis. Each filled circle represents the p value of analyzed variants (as -$\log_{10}$ p values) plotted against their physical position (NCBI Build 37). The p value at the index variant is represented by a purple circle. The other variants in the region are colored depending on their degree of correlation (r^2) with the index variant according to a scale from r^2 = 0 (blue) to r^2 = 1 (red). Gene location is annotated based on the UCSC genome browser.

a suggestive association of several genetic loci with susceptibility to osteolysis and with time to revision in those patients with osteolysis. The largest association block in the case-control meta-analysis centered on the gene encoding DPY19L2 pseudogene 3 on the short arm of chromosome 7 (intronic variant rs850092). This gene has

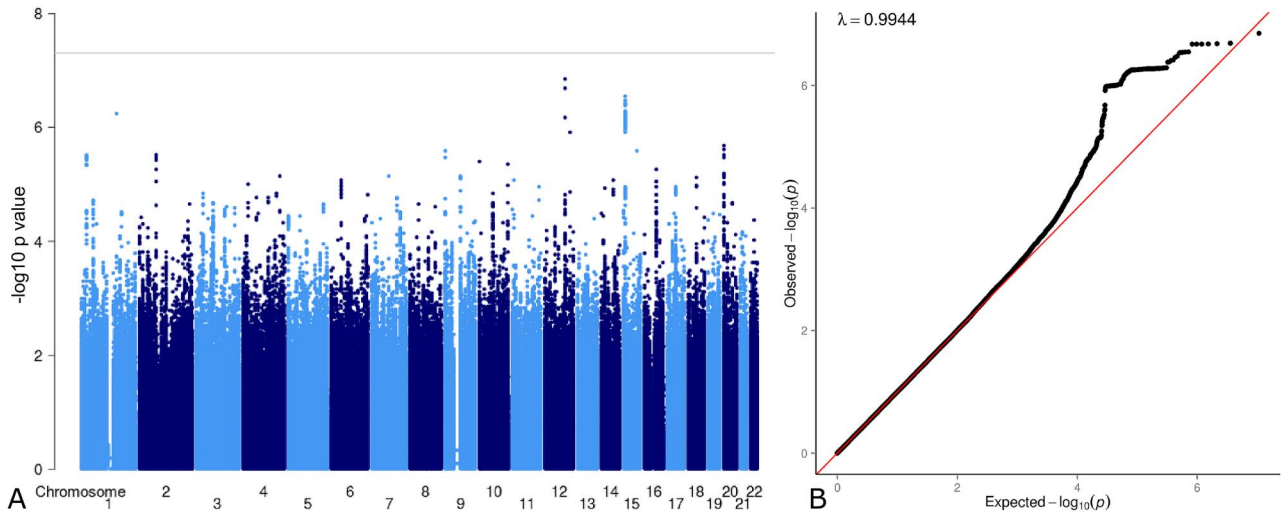

Fig. 7 A-B (**A**) Manhattan plot of the Norwegian and UK cohort time-to-revision association meta-analysis in osteolysis patients only (n = 1096) showing the -log10 p values for each variant (y-axis) plotted against their respective chromosomal position (x-axis) and illustrating 11 independent genetic association peaks. (**B**) Graph showing QQ plot of the p values for the Norwegian cohort time-to-revision association analysis, where the x-axis indicates the expected −log10 p values and the y-axis the observed ones. The red line represents the null hypothesis of no association at any locus and λ is the genomic inflation factor.

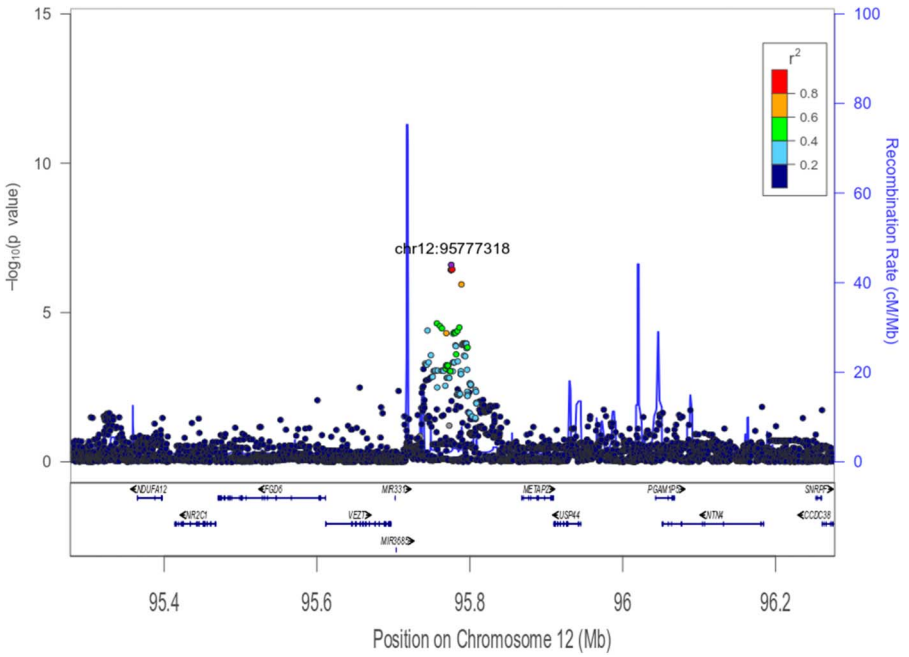

Fig. 8 Regional association plot showing the lead time to prosthesis revision signal at rs10507055 in the association meta-analysis. Each filled circle represents the p value of analyzed variants (as -$\log_{10}$ p values) plotted against their physical position (NCBI Build 37). The p value at the index variant is represented by a purple circle. The other variants in the region are colored depending on their degree of correlation (r^2) with the index variant according to a scale from $r^2 = 0$ (blue) to $r^2 = 1$ (red). Gene location is annotated based on the UCSC genome browser.

not been characterized previously and its function is unknown. However, this signal also lies adjacent to microRNA 550a-3 (MiR550A3). MicroRNAs are a recently discovered group of RNAs that function to regulate the production of other peptides and are currently being explored as putative biomarkers and treatments for musculoskeletal and other diseases [4, 6, 39, 42]. In our quantitative trait meta-analysis of time-to-prosthesis revision, we identified a large block of 174 correlated variants in chromosome 15. The lead signal for this block was intronic variant rs12899987 that lies within the gene encoding OTU deubiquitinase 7A (OTUD7A). OTUD7A is an intracellular enzyme that modulates NFκB signaling through TRAF6 that is pivotal in proinflammatory cytokine signaling in periprosthetic osteolysis [55] and represents a potentially actionable target in its prevention [29]. The most statistically significant signal in the time-to-revision analysis lies within the gene LOC105369917. The function of this gene has not been explored. However, this signal also lies adjacent to two further microRNA sites, MiR331 and MiR3685. Further exploration by fine mapping of these loci is required to identify the causal variants at each signal.

This study has several limitations. Although these cohorts represent a nationwide and a large regional cohort purposely collected for the study of osteolysis genetics, the sample sizes remain small compared with other population-based genomic studies [10, 50, 57]. For the case-control analysis, we had > 80% power to detect ORs of 1.5 to 1.9 for variants with MAF 5% to 15% using the combined sample size and combined case-control ratio (1:2.2). For the continuous trait, we assumed a population mean of 0 and a SD of 1. The combined sample size had > 80% power to detect variants at genome-wide significance ($p < 5 \times 10^{-8}$) with a modest effect size (beta of 1.3 to 1.5) for common variants (MAF, 0.5 to 0.15) and a moderate effect size (beta of 1.6 to 2.0) for variants with MAF 0.14 to 0.05. However, similar sample sizes have been used previously to identify the genetic underpinnings in other complex musculoskeletal diseases, including the association of Wnt signaling with Dupuytren's disease (n = 960 cases) [8].

The case-ascertainment approach also differed between the cohorts. The UK participants were recruited face to face using the primary hospital record and included radiographic evidence of osteolysis or the revision operative record, as described previously, and comprised all patients who fit the relevant inclusion and exclusion criteria. The known epidemiologic risk factors for osteolysis were therefore also reflected in the UK study population.

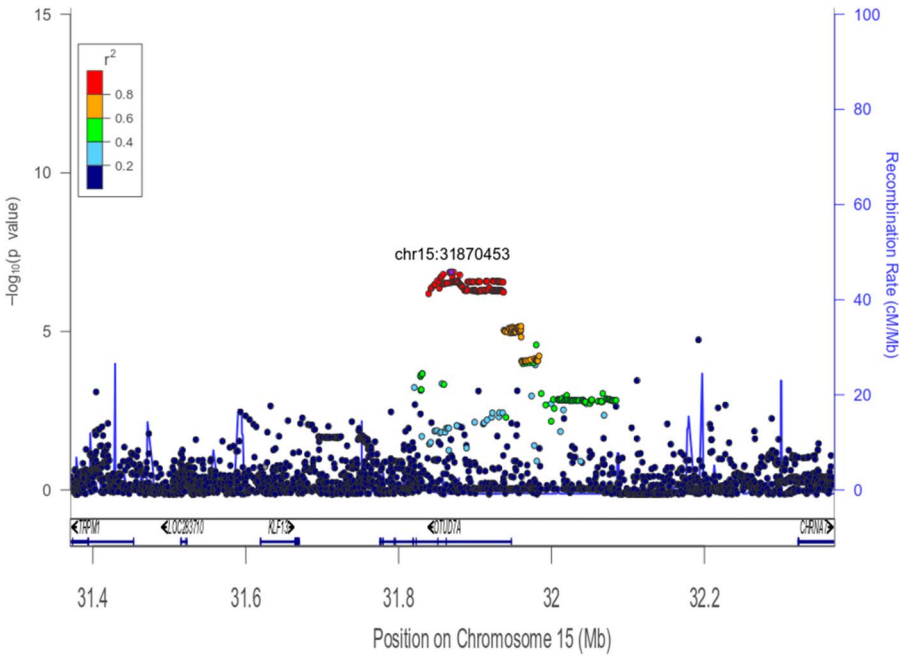

Fig. 9 Regional association plot showing the lead time to revision signal at rs12899987 in the association meta-analysis. Each filled circle represents the p value of analyzed variants (as -$\log_{10}$ p values) plotted against their physical position (NCBI Build 37). The p value at the index variant is represented by a purple circle. The other variants in the region are colored depending on their degree of correlation (r^2) with the index variant according to a scale from $r^2 = 0$ (blue) to $r^2 = 1$ (red). Gene location is annotated based on the UCSC genome browser.

Although this provides evidence for the generalizability of our findings to other populations, we had to adjust for these covariates in the genetic association analyses. All analyses are shown postadjustment for these clinical risk factors as well as for an unidentified population and analytical stratification using principal component and regression analysis. The Norwegian cohort study design and recruitment used the Norwegian Arthroplasty Register as the source data set with documented revision events and indications recorded by the operating surgeon. The patients with osteolysis were recruited before the control patients and at a control:case ratio of 3:1 with screening to match the demographic characteristics and implant type and operation year (± 2 years) as closely as possible. This approach allowed the recruitment of a large case-control matched population but increases the risk of ascertainment bias because patients with silent osteolysis may have been recorded as controls and cases could have been misdiagnosed. Despite this approach, small statistical differences in population demographics remained because of the large sample sizes involved, although their clinical relevance may be limited. For example, the mean age at primary operation in the Norwegian control patients was 66 years, whereas it was 64 years in the patients with osteolysis. We adjusted for these residual differences in the Norwegian cohort association analysis in the same manner as we did for the UK cohort.

We found more genetic signals within the UK versus the Norwegian population despite the smaller cohort size. These differences may be genuine. The observed differences might also reflect differences in case ascertainment or other unknown biases between the cohorts. However, the population-level genomic architecture of both cohorts by variant allele frequency was similar, indicating no significant genetic heterogeneity between the cohorts. The study participants in each cohort also came from different healthcare economies with potential differences in diagnostic and treatment thresholds. Individual surgeons' clinical practices also differ, resulting in management variation both between and within the cohorts. However, these types of classification differences are unlikely to map to particular genotypes, and thus their likely effect is to create noise limiting the ability of the study to detect genuine genetic signals rather than increasing the false-positive discovery rate.

We, and others, have previously shown the association of osteolysis with variation in several candidate genes [11, 30]. In these studies, the genes are selected based on their known biologic function or a previous association of the selected variants with other diseases that share biologic similarities. The threshold for statistical significance is also

set low (typically at $p < 0.05$), favoring the identification of a positive association. Although these discovery studies lend support to the concept of a disease driven by heritable variation, these associations commonly are not reproduced when examined in independent cohorts [46], and the overall contribution of genetic variation to the disease remains unanswered. In contrast, genome-wide studies allow examination of the overall genetic architecture of the disease that underpins the differences in susceptibility between individuals. However, these studies require larger sample sizes and are accompanied by substantially more stringent thresholds for significance.

The data presented here suggest the association of several previously unstudied genomic loci with osteolysis. The observations that such loci may reside within areas of the genome about which we still know very little provide the opportunity for novel avenues for exploration of the disease. However, further replication of the observed associations is required to confirm their validity, fine-mapping to precisely localize causal associations, and experimental study of their biologic function will enable us to clearly understand their role in osteolysis biology and to translate this new knowledge into diagnostic and therapeutic tools.

Acknowledgments We thank the Norwegian Arthroplasty Register patients and staff and the South Yorkshire and North Derbyshire Musculoskeletal Biobank for facilitating these studies.

References

1. Ambruzova Z, Gallo J, Mrazek F, Kubistova Z, Onderkova J, Petrek M. Association of cytokine gene polymorphisms with expansile periprosthetic osteolysis in total hip arthroplasty. *Tiss Antigens.* 2006;67:528–528.
2. Bachmann HS, Hanenkamp S, Kornacki B, Frey UH, Bau M, Siffert W, Wedemeyer C. Gender-dependent association of the GNAS1 T393C polymorphism with early aseptic loosening after total hip arthroplasty. *J Orthop Res.* 2008;26:1562–1568.
3. Bi Y, Seabold JM, Kaar SG, Ragab AA, Goldberg VM, Anderson JM, Greenfield EM. Adherent endotoxin on orthopedic wear particles stimulates cytokine production and osteoclast differentiation. *J Bone Miner Res.* 2001;16:2082–2091.
4. Budd E, Waddell S, de Andres MC, Oreffo ROC. The potential of microRNAs for stem cell-based therapy for degenerative skeletal diseases. *Curr Mol Biol Rep.* 2017;3:263–275.
5. Burton L, Paget D, Binder NB, Bohnert K, Nestor BJ, Sculco TP, Santambrogio L, Ross FP, Goldring SR, Purdue PE. Orthopedic wear debris mediated inflammatory osteolysis is mediated in part by NALP3 inflammasome activation. *J Orthop Res.* 2013;31:73–80.
6. Chakraborty C, Sharma AR, Sharma G, Sarkar BK, Lee SS. The novel strategies for next-generation cancer treatment: miRNA combined with chemotherapeutic agents for the treatment of cancer. *Oncotarget.* 2018;9:10164–10174.
7. Clohisy JC, Frazier E, Hirayama T, Abu-Amer Y. RANKL is an essential cytokine mediator of polymethylmethacrylate particle-induced osteoclastogenesis. *J Orthop Res.* 2003;21:202–212.
8. Dolmans GH, Werker PM, Hennies HC, Furniss D, Festen EA, Franke L, Becker K, van der Vlies P, Wolffenbuttel BH, Tinschert S, Toliat MR, Nothnagel M, Franke A, Klopp N, Wichmann HE, Nurnberg P, Giele H, Ophoff RA, Wijmenga C; Dutch Dupuytren Study Group; German Dupuytren Study Group; LifeLines Cohort Study; BSS-Godd Consortium. Wnt signaling and Dupuytren's disease. *N Engl J Med.* 2011;365:307–317.
9. Doro C, Dimick J, Wainess R, Upchurch G, Urquhart A. Hospital volume and inpatient mortality outcomes of total hip arthroplasty in the United States. *J Arthroplasty.* 2006;21:10–16.
10. Flint J, Munafo M. Schizophrenia: genesis of a complex disease. *Nature.* 2014;511:412–413.
11. Gallo J, Mrazek F, Petrek M. Variation in cytokine genes can contribute to severity of acetabular osteolysis and risk for revision in patients with ABG 1 total hip arthroplasty: a genetic association study. *BMC Med Genet.* 2009;10:109.
12. Gordon A, Greenfield EM, Eastell R, Kiss-Toth E, Wilkinson JM. Individual susceptibility to periprosthetic osteolysis is associated with altered patterns of innate immune gene expression in response to pro-inflammatory stimuli. *J Orthop Res.* 2010;28:1127–1135.
13. Gordon A, Kiss-Toth E, Stockley I, Eastell R, Wilkinson JM. Polymorphisms in the interleukin-1 receptor antagonist and interleukin-6 genes affect risk of osteolysis in patients with total hip arthroplasty. *Arthritis Rheum.* 2008;58:3157–3165.
14. Gordon A, Southam L, Loughlin J, Wilson AG, Stockley I, Hamer AJ, Eastell R, Wilkinson JM. Variation in the secreted frizzled-related protein-3 gene and risk of osteolysis and heterotopic ossification after total hip arthroplasty. *J Orthop Res.* 2007;25:1665–1670.
15. Harris WH. Wear and periprosthetic osteolysis: the problem. *Clin Orthop Relat Res.* 2001;393:66–70.
16. Harris WH, McGann WA. Loosening of the femoral component after use of the medullary-plug cementing technique. Follow-up note with a minimum five-year follow-up. *J Bone Joint Surg Am.* 1986;68:1064–1066.
17. Harris WH, Penenberg BL. Further follow-up on socket fixation using a metal-backed acetabular component for total hip replacement. A minimum ten-year follow-up study. *J Bone Joint Surg Am.* 1987;69:1140–1143.
18. Havelin LI, Engesaeter LB, Espehaug B, Furnes O, Lie SA, Vollset SE. The Norwegian Arthroplasty Register: 11 years and 73,000 arthroplasties. *Acta Orthop.* 2000;71:337–353.
19. Haynes DR, Crotti TN, Potter AE, Loric M, Atkins GJ, Howie DW, Findlay DM. The osteoclastogenic molecules RANKL and RANK are associated with periprosthetic osteolysis. *J Bone Joint Surg Br.* 2001;83:902–911.
20. Holding CA, Findlay DM, Stamenkov R, Neale SD, Lucas H, Dharmapatni AS, Callary SA, Shrestha KR, Atkins GJ, Howie DW, Haynes DR. The correlation of RANK, RANKL and TNFalpha expression with bone loss volume and polyethylene wear debris around hip implants. *Biomaterials.* 2006;27:5212–5219.
21. Holt G, Murnaghan C, Reilly J, Meek RM. The biology of aseptic osteolysis. *Clin Orthop Relat Res.* 2007;460:240–252.
22. Hopper RH Jr, Ho H, Sritulanondha S, Williams AC, Engh CA Jr. Otto Aufranc Award: Crosslinking reduces THA wear, osteolysis, and revision rates at 15-year followup compared with noncrosslinked polyethylene. *Clin Orthop Relat Res.* 2018;476:279–290.
23. International HapMap Consortium. A haplotype map of the human genome. *Nature.* 2005;437:1299–1320.
24. Jiranek WA, Machado M, Jasty M, Jevsevar D, Wolfe HJ, Goldring SR, Goldberg MJ, Harris WH. Production of cytokines around loosened cemented acetabular components. Analysis with immunohistochemical techniques and in situ hybridization. *J Bone Joint Surg Am.* 1993;75:863–879.

25. Kadoya Y, Kobayashi A, Ohashi H. Wear and osteolysis in total joint replacements. *Acta Orthop Suppl.* 1998;278:1–16.
26. Kolundzic R, Orlic D, Trkulja V, Pavelic K, Troselj KG. Single nucleotide polymorphisms in the interleukin-6 gene promoter, tumor necrosis factor-alpha gene promoter, and transforming growth factor-beta1 gene signal sequence as predictors of time to onset of aseptic loosening after total hip arthroplasty: preliminary study. *J Orthop Sci.* 2006;11:592–600.
27. Koulouvaris P, Ly K, Ivashkiv LB, Bostrom MP, Nestor BJ, Sculco TP, Purdue PE. Expression profiling reveals alternative macrophage activation and impaired osteogenesis in periprosthetic osteolysis. *J Orthop Res.* 2008;26:106–116.
28. Labek G, Thaler M, Janda W, Agreiter M, Stockl B. Revision rates after total joint replacement: cumulative results from worldwide joint register datasets. *J Bone Joint Surg Br.* 2011;93:293–297.
29. Lin TH, Pajarinen J, Lu L, Nabeshima A, Cordova LA, Yao Z, Goodman SB. NF-kappaB as a therapeutic target in inflammatory-associated bone diseases. *Adv Protein Chem Struct Biol.* 2017;107:117–154.
30. MacInnes SJ, Del Vescovo E, Kiss-Toth E, Ollier WE, Kay PR, Gordon A, Greenfield EM, Wilkinson JM. Genetic variation in inflammatory and bone turnover pathways and risk of osteolytic responses to prosthetic materials. *J Orthop Res.* 2015;33:193–198.
31. Mahomed NN, Barrett JA, Katz JN, Phillips CB, Losina E, Lew RA, Guadagnoli E, Harris WH, Poss R, Baron JA. Rates and outcomes of primary and revision total hip replacement in the United States medicare population. *J Bone Joint Surg Am.* 2003;85:27–32.
32. Makela KT, Matilainen M, Pulkkinen P, Fenstad AM, Havelin L, Engesaeter L, Furnes O, Pedersen AB, Overgaard S, Karrholm J, Malchau H, Garellick G, Ranstam J, Eskelinen A. Failure rate of cemented and uncemented total hip replacements: register study of combined Nordic database of four nations. *BMJ.* 2014;348:f7592.
33. Malik MH, Bayat A, Jury F, Kay PR, Ollier WE. Genetic susceptibility to total hip arthroplasty failure–positive association with mannose-binding lectin. *J Arthroplasty.* 2007;22:265–270.
34. Malik MH, Bayat A, Jury F, Ollier WE, Kay PR. Genetic susceptibility to hip arthroplasty failure–association with the RANK/OPG pathway. *Int Orthop.* 2006;30:177–181.
35. Malik MH, Jury F, Bayat A, Ollier WE, Kay PR. Genetic susceptibility to total hip arthroplasty failure: a preliminary study on the influence of matrix metalloproteinase 1, interleukin 6 polymorphisms and vitamin D receptor. *Ann Rheum Dis.* 2007;66:1116–1120.
36. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007;39:906–913.
37. Matthews JB, Green TR, Stone MH, Wroblewski BM, Fisher, Ingham E. Comparison of the response of primary human peripheral blood mononuclear phagocytes from different donors to challenge with model polyethylene particles of known size and dose. *Biomaterials.* 2000;21:2033–2044.
38. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM, Fuchsberger C, Danecek P, Sharp K, Luo Y, Sidore C, Kwong A, Timpson N, Koskinen S, Vrieze S, Scott LJ, Zhang H, Mahajan A, Veldink J, Peters U, Pato C, van Duijn CM, Gillies CE, Gandin I, Mezzavilla M, Gilly A, Cocca M, Traglia M, Angius A, Barrett JC, Boomsma D, Branham K, Breen G, Brummett CM, Busonero F, Campbell H, Chan A, Chen S, Chew E, Collins FS, Corbin LJ, Smith GD, Dedoussis G, Dorr M, Farmaki AE, Ferrucci L, Forer L, Fraser RM, Gabriel S, Levy S, Groop L, Harrison T, Hattersley A, Holmen OL, Hveem K, Kretzler M, Lee JC, McGue M, Meitinger T, Melzer D, Min JL, Mohlke KL, Vincent JB, Nauck M, Nickerson D, Palotie A, Pato M, Pirastu N, McInnis M, Richards JB, Sala C, Salomaa V, Schlessinger D, Schoenherr S, Slagboom PE, Small K, Spector T, Stambolian D, Tuke M, Tuomilehto J, Van den Berg LH, Van Rheenen W, Volker U, Wijmenga C, Toniolo D, Zeggini E, Gasparini P, Sampson MG, Wilson JF, Frayling T, de Bakker PI, Swertz MA, McCarroll S, Kooperberg C, Dekker A, Altshuler D, Willer C, Iacono W, Ripatti S, Soranzo N, Walter K, Swaroop A, Cucca F, Anderson CA, Myers RM, Boehnke M, McCarthy MI, Durbin R; Haplotype Reference Consortium. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet.* 2016;48:1279–1283.
39. Nguyen LT, Sharma AR, Chakraborty C, Saibaba B, Ahn ME, Lee SS. Review of prospects of biological fluid biomarkers in osteoarthritis. *Int J Mol Sci.* 2017;18. pii: E601. doi: 10.3390/ijms18030601.
40. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. *Genet Epidemiol.* 2008;32:381–385.
41. Pearl JI, Ma T, Irani AR, Huang Z, Robinson WH, Smith RL, Goodman SB. Role of the Toll-like receptor pathway in the recognition of orthopedic implant wear-debris particles. *Biomaterials.* 2011;32:5535–5542.
42. Roitbak T. Silencing a Multifunctional microRNA is beneficial for stroke recovery. *Front Mol Neurosci.* 2018;11:58.
43. Sadoghi P, Liebensteiner M, Agreiter M, Leithner A, Bohler N, Labek G. Revision surgery after total joint arthroplasty: a complication-based analysis using worldwide arthroplasty registers. *J Arthroplasty.* 2013;28:1329–1332.
44. Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total hip arthroplasty. Polyethylene wear debris and the concept of the effective joint space. *J Bone Joint Surg Am.* 1992;74:849–863.
45. Schmalzried TP, Shepherd EF, Dorey FJ, Jackson WO, dela RM, Fa'vae F, McKellop HA, McClung CD, Martell J, Moreland JR, Amstutz HC. The John Charnley Award. Wear is a function of use, not time. *Clin Orthop Relat Res.* 2000;381:36–46.
46. Stelmach P, Kauther MD, Fuest L, Kurscheid G, Gehrke T, Klenke S, Jager M, Wedemeyer C, Bachmann HS. Relationship between GNAS1 T393C polymorphism and aseptic loosening after total hip arthroplasty. *Eur J Med Res.* 2017;22:29.
47. Stelmach P, Wedemeyer C, Fuest L, Kurscheid G, Gehrke T, Klenke S, Jager M, Kauther MD, Bachmann HS. The BCL2 -938C>A promoter polymorphism is associated with risk for and time to aseptic loosening of total hip arthroplasty. *PloS One.* 2016;11:e0149528.
48. Takagi M, Tamaki Y, Hasegawa H, Takakubo Y, Konttinen L, Tiainen VM, Lappalainen R, Konttinen YT, Salo J. Toll-like receptors in the interface membrane around loosening total hip replacement implants. *J Biomed Mater Res A.* 2007;81:1017–1026.
49. Tsao AK, Jones LC, Lewallen DG. What patient and surgical factors contribute to implant wear and osteolysis in total joint arthroplasty? *J Am Acad Orthop Surg.* 2008;16(Suppl 1):S7–13.
50. Van Cauwenberghe C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med.* 2016;18:421–430.
51. Wedemeyer C, Kauther MD, Hanenkamp S, Nuckel H, Bau M, Siffert W, Bachmann HS. BCL2-938C>A and CALCA-1786T>C polymorphisms in aseptic loosened total hip arthroplasty. *Eur J Med Res.* 2009;14:250–255.
52. Westacott CI, Taylor G, Atkins R, Elson C. Interleukin 1 alpha and beta production by cells isolated from membranes around

aseptically loose total joint replacements. *Ann Rheum Dis.* 1992; 51:638–642.
53. Wilkinson JM, Wilson AG, Stockley I, Scott IR, Macdonald DA, Hamer AJ, Duff GW, Eastell R. Variation in the TNF gene promoter and risk of osteolysis after total hip arthroplasty. *J Bone Miner Res.* 2003;18:1995–2001.
54. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* 2010; 26:2190–2191.
55. Xu J, Wu HF, Ang ES, Yip K, Woloszyn M, Zheng MH, Tan RX. NF-kappaB modulators in osteolytic bone diseases. *Cytokine Growth Factor Rev.* 2009;20:7–17.
56. Xu JW, Konttinen YT, Lassus J, Natah S, Ceponis A, Solovieva S, Aspenberg P, Santavirta S. Tumor necrosis factor-alpha (TNF-alpha) in loosening of total hip replacement (THR). *Clin Exp Rheumatol.* 1996;14:643–648.
57. Zengini E, Hatzikotoulas K, Tachmazidou I, Steinberg J, Hartwig FP, Southam L, Hackinger S, Boer CG, Styrkarsdottir U, Gilly A, Suveges D, Killian B, Ingvarsson T, Jonsson H, Babis GC, McCaskie A, Uitterlinden AG, van Meurs JBJ, Thorsteinsdottir U, Stefansson K, Davey Smith G, Wilkinson JM, Zeggini E. Genome-wide analyses using UK Biobank data provide insights into the genetic architecture of osteoarthritis. *Nat Genet.* 2018 Mar 20. [Epub ahead of print]
58. Zhan C, Kaczmarek R, Loyo-Berrios N, Sangl J, Bright RA. Incidence and short-term outcomes of primary and revision hip replacement in the United States. *J Bone Joint Surg Am.* 2007;89: 526–533.