Whole genome sequencing identifies rare genotypes in *COMP* and *CHADL* associated with high risk of hip osteoarthritis

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Abstract

We performed a genome-wide association study of total hip replacements, based on variants identified through whole-genome sequencing, which included 4,657 Icelandic patients and 207,514 population controls. We discovered two rare signals that strongly associate with osteoarthritis total hip replacement; a missense mutation, c.1141G>C (p.Asp369His), in the *COMP* gene (allelic frequency = 0.026%, $P = 4.0 \times 10^{-12}$, OR = 16.7), and a frameshift mutation, rs532464664 (p.Val330GlyfsTer106) in the *CHADL* gene that associates through a recessive mode of inheritance (homozygote frequency = 0.15%, $P = 4.5 \times 10^{-18}$ and OR = 7.71). c.1141G>C heterozygotes and individuals homozygous for rs532464664 have their hip replacement operation 13.5 years and 4.9 years younger than others ($P = 0.0020$ and $P = 0.0026$), respectively. We show that the full-length *CHADL* transcript is expressed in cartilage. Furthermore, the premature stop codon introduced by the *CHADL* frameshift mutation results in nonsense mediated decay of the mutated transcripts.

Osteoarthritis (OA) is a highly heterogeneous disease, or a set of disorders with a great impact on the quality of life. OA is a disease of the entire joint, affecting cartilage, synovium, subchondral bone, ligaments and the joint capsule[1]. Total hip replacement (THR) is a treatment for severe OA of the hip, indicated by severe pain and reduced mobility, although radiographic changes in these subjects may vary. Genome-wide association studies (GWAS) of hip OA have reported a number of common sequence variants, all conferring small to moderate effects[2-8]. Here, we report on a GWAS yielding two rare sequence variants that associate with end-stage hip OA; THR.

We included 4,657 Icelanders who have undergone THR because of OA as cases and 207,514 individuals as population controls in the GWAS. The sequence variants included in the analyses were identified through whole-genome sequencing of 8,453 Icelanders, and subsequently imputed into those 150,656 who had been chip-typed and their first and second degree relatives. We then tested for association between THR and these sequence variants; 31.6 million variants under the multiplicative model, and 19.2 million variants under the recessive model.

Under the multiplicative model, two variants met the thresholds of genome-wide significance that accounts for prior probability of impact of the variants[9] (**Methods, Supplementary Fig. 1a**); a rare missense variant in the cartilage oligomeric matrix protein (*COMP*) gene at 19p13.11 (hg38 position chr19:18,787,521, $P = 3.1 \times 10^{-9}$, OR = 10.4, allelic frequency 0.033%) and a frameshift variant in the chondroadherin-like (*CHADL*) gene at 22q13.2 (rs532464664, $P = 1.5 \times 10^{-7}$, OR = 1.37, allelic frequency 3.92%). Variants at other loci did not reach genome-wide significance. Nominal association ($P < 0.05$) was observed for 9 of the 12 previously reported hip OA loci (**Supplementary Table 1**).

Because the mutation in *COMP* is very rare in our dataset it was validated by direct genotyping. The directly typed genotypes were then used to re-impute the variant into the OA cases and controls (**Supplementary Note**). This resulted in improved imputation (imputation information 1.00 versus 0.95) and stronger association ($P = 4.0 \times 10^{-12}$, OR = 16.7, allelic frequency 0.026% or in 1 in 1900 persons in our study population). (**Table 1, Supplementary Table 2**).

The variant in the *COMP* gene is a C to G transversion at hg38 position chr19:18,787,521 (NM_000095.2:c.1141G>C, NP_000086.2:p.Asp369His), resulting in a replacement of asparagine with histidine at amino acid position 369 of the COMP protein (**Supplementary Fig. 2**). The COMP protein is an important functional component of the extracellular matrix of the cartilage[10] and serum level of the protein is an indicator of cartilage breakdown. As such it is being evaluated as a prognostic marker for incidence of knee and hip OA[11]. Missense mutations in the COMP protein are known to cause two skeletal dysplasias; pseudoachondroplasia (PSACH)[12] and multiple epiphyseal dysplasia (MED)[13] (**Supplementary Note**, **Supplementary Fig. 2**), conditions characterized by severe to mild short limb dwarfism with early onset OA because of dysplasia under a dominant mode of inheritance. None of the c.1141G>C heterozygotes (n = 117) have been diagnosed with MED or other dysplasia, and are of normal height (**Supplementary Fig. 3, Supplementary Table 3**). Their disease is indistinguishable from other primary hip OA[14] (**Supplementary Note, Supplementary Fig. 4**). The *COMP* c.1141G>C heterozygous individuals form a genealogical cluster that spans seven generations and track the mutation to a founder born around 1840 (**Supplementary Figs. 5, 6**). The c.1141G>C mutation also associates with radiographic OA without THR; $P = 8.1 \times 10^{-20}$ and OR = 29.6 when THR and radiographic OA are analyzed together (5,109 cases / 222,460 controls). The mutation also

associates with knee, spine and hand OA, albeit with weaker effects (**Supplementary Table 2**). Furthermore, we found that the c.1141G>C heterozygotes are 13.5 years younger than others to have their hip replaced, or 54.5 years old, on average, versus 68.0 years old (P = 0.0020, **Fig. 1a, 1b, Supplementary Table 4**). Heterozygous carriers of the mutation in the control group are also younger than the THR heterozygotes (**Supplementary Table 5**).The variant is not reported in the Exome Aggregation Consortium (ExAC) Browser[15], the Genome Aggregation Database (gnomAD)[15], or in the Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/v.0.0.17) and was not found in samples of European origin (n = 12,980) whom we directly genotyped. Thus, the variant likely represents a single mutational event that predisposes to OA at the mild end of the *COMP* gene mutations spectrum and follows a dominant mode of inheritance. See **Supplementary Note** for more data on the c.1141G>C (p.Asp369His) variant.

The association of the rs532464664 frameshift variant in the *CHADL* gene with THR is much stronger under the recessive than the multiplicative model (**Supplementary Fig. 1b**), reaching $P = 4.5 \times 10^{-18}$ and OR = 7.7 (**Table 1**). Furthermore, the associated risk in the multiplicative model can be fully explained by the contribution of the homozygous individuals. The frameshift variant is an 8 base pair (bp) insert, rs532464664, in the third exon of the gene (NM_138481.1:c.988_989insGGCGCGCG, NP_612490.1:p.Val330GlyfsTer106, allele frequency 3.92%, homozygous frequency 0.15%), that puts the protein out of frame at amino acid 330, out of the 762 full length protein, and introduces a stop codon 106 amino acids downstream of the frameshift (**Supplementary Fig. 7**). Under the recessive model eight additional variants at the *CHADL* locus, all highly correlated with the frameshift mutation, associate with THR. None of

these variants associate significantly after accounting for the effect of the homozygous state of the rs532464664[insGGCGCGCG] allele (**Fig. 2, Supplementary Fig. 8**, **Supplementary Table 6**). Similar to the variant in *COMP*, we found that the rs532464664 mutation affects the age at THR; homozygous individuals were, on average, 4.9 years younger than others when they had their hip replacement (P = 0.0073; **Fig. 1c**, **1d**, **Supplementary Table 7**). Homozygous individuals in the control group are also younger than those in the THR group (**Supplementary Table 5**). We re-examined all available radiographs of the hip for 784 of the homozygous individuals and demonstrated that their disease is indistinguishable from other primary hip OA (**Supplementary Note, Supplementary Fig. 9**).

The rs532464664 is in a region with very high GC content (80%) that is badly covered by the standard Illumina TruSeq sequencing technique. This may explain why the mutation is not reported in the ExAC[15], gnomAD[15], or EVS databases (**Supplementary Table 8, Supplementary Fig. 10**). However, since a large fraction of our sequencing data is generated with either a Illumina PCR free TruSeq or Illumina TruSeq Nano methods that perform better than the standard TruSeq method in GC-rich regions, we are able to reliably call rs532464664 (**Supplementary Fig. 11**).

We directly genotyped the rs532464664 variant in ten different populations, four of which included THR samples (**Methods**). Rs532464664 is present in all the populations except the Koreans, with a frequency range from 0.13% to 2.55% (**Supplementary Table 9**).

We found five homozygous individuals who had undergone THR in UK samples (N_{THR} = 2,711), one in Danish samples (N_{THR} = 660), one in Norwegian samples (N_{THR} = 138) and none in Swedish samples (N_{THR} = 376). Due to the low frequency of homozygous individuals in these samples

(0.041% to 0.065%) we lack statistical power to assess association with THR. However, we observed, like in Iceland, a similar, although non-significant (P = 0.081), trend of THR at a younger age for the homozygous UK individuals; the UK homozygotes were 59 years old compared to 67 years (**Fig. 1e**).

We explored mRNA expression of the *CHADL* gene in RNA sequencing data of white blood cells (n = 2,528), adipose tissue (n = 655) and cartilage (n = 16) and identified two types of *CHADL* transcripts; a full-length transcript in cartilage and a shorter non-coding transcript, with start site in exon 3 downstream of rs532464664, in adipose tissue (**Supplementary Fig. 12**). In adipose tissue rs532464664 is correlated with increased expression of this shorter transcript ($P = 1.4 \times 10^{-23}$, 54% increase in expression per allele carried) (**Supplementary Fig. 13**).

We further explored *CHADL* mRNA expression profile in 23 tissues by quantitative PCR (qPCR) (**Methods**) and found that highest expression of full-length *CHADL* is in the spinal cord and brain, followed by cartilage tissue (**Supplementary Fig. 14**). Specific qPCR analysis of joint tissue samples showed highest expression of full-length *CHADL* in joint cartilage (**Fig. 3a**) and in cultured chondrocytes (**Fig. 3b**, **3c**). The high expression of the *CHADL* gene in cartilage is in line with expression of the mouse homolog, *Chadl*[16].

Since no carriers of rs532464664 were among the RNA sequenced cartilage samples we could not determine whether the premature stop codon introduced by rs532464664 induced nonsense mediated decay (NMD), a process responsible for eliminating aberrant mRNA transcripts with premature stop codons[17]. To investigate potential NMD effect of the premature stop codon we utilized an *in vitro* reporter system for evaluation of NMD (**Methods,**

Supplementary Fig. 15). Similar systems have been used successfully to monitor the status of the NMD machinery in cells[18,19]. Strikingly, the expression of the reporter protein was reduced when fused to a gene fragment harboring rs532464664[insGGCGCGCG] in comparison to the wild type allele and this reduction was partially reverted with the NMD inhibitors, indicating that rs532464664 induces NMD of the aberrant mRNA (**Supplementary Fig. 15**). *Chadl* knockdown in the mouse chondrogenic cell-line (ATCD5) demonstrated that lack of *Chadl* enhances the differentiation of the cell line and deposition of collagen II and aggrecan. This links the protein with the formation of extracellular matrix of the cartilage and suggests that *Chadl* may play a negative regulatory role in the cartilage.

In summary, we report a rare missense variant in the *COMP* gene and a recessive frameshift mutation in the *CHADL* gene that associates strongly with OA hip replacement. Both variants confer substantial risk of THR; 16.7 and 7.7, respectively. These odds ratios are substantially higher than for the previously reported common OA variants, which range from 1.1 to 1.79[7], and may represent Mendelian forms of OA. Furthermore, both those homozygous for rs532464664 (p.Val330GlyfsTer106) and those heterozygous for the c.1141G>C (p.Asp369His) mutation in *COMP* have hip replacement at a younger age than others. Although the *COMP* mutation is specific to the Icelandic population, the frameshift mutation in *CHADL* is widespread in populations of European and Middle-Eastern descent and found in lower frequency in East-Asians, suggesting that the mutation occurred a long time ago. It is one of very few variants that associate under the recessive model in GWAS of a common disease.

Data availability. The Icelandic population WGS data has been deposited at the European Variant Archive under accession code PRJEB15197. The authors declare that the data supporting the findings of this study are available within the article, its supplementary Information files and on request.

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Author contributions: The study was designed and results were interpreted by U.S., H.H., U.T., P.S., J.L. and K.S. Phenotype data, recruitment and coordination of Icelandic subjects were managed by T.I., H.J. and U.S. Subject ascertainment, recruitment, management and coordination of samples from non-Icelandic populations was done by O.A.A., I.S.A., A.B., T.F., G.S., H.S., C.C., L.S.L., I.J., N.S., P.B., A. Helgadottir, U.T., J.L., arcOGEN consortium, L.K., J.M., T.R., C.W., F.H., F.A., M.D., N.L.S.T., J.M.K. and U.S. T.I analyzed hip radiographs. H.S.J. and O.M. performed the genotyping. A.S., A.J., A.B.A., L.N.R., A.V., J.L., G.H.H. and HH carried out and analyzed the expression experiments. G.L.N. and A.B.A. designed and performed the NMD experiments. G.M., O.M., A.O., G. Sveinbjornsson, F.Z., G.S., A. Helgason, A.K., D.G., and P.S. did the bioinformatics analysis, whole genome sequencing, genealogy, imputation and association analysis in the Icelandic data set. All authors contributed to the final version of the manuscript.

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FIGURE LEGENDS

Figure 1. Age at total hip replacement by *COMP* variant genotype and *CHADL* variant genotype

a) Age at THR by *COMP* variant c.1141G>C (g.chr19:18,787,521) genotype.
b) Kaplan-Meier estimator curves for THR according to genotype status of c.1141G>C in *COMP*. Hazard ratio for heterozygotes vs. non-carriers is 9.46 (95% CI: 5.09 – 17.61).
c) Age at THR by *CHADL* variant rs532464664 genotype. Mann-Whitney tests for age at operation give $P = 0.0025$ for homozygotes vs. non-carriers, $P = 0.35$ for heterozygotes vs. non-carriers and $P = 0.0080$ for homozygotes vs. heterozygotes.
d) Kaplan-Meier curves for THR according to genotype status of rs532464664 in *CHADL*. Hazard ratio for homozygotes versus the two other genotypes is 8.20 (95% CI: 5.93 – 11.34)
e) Age at THR by *CHADL* rs532464664 variant genotype for the UK arcOGEN cohort.

In the Icelandic samples all individuals were chip-typed and the genotypes imputed, in the UK samples the genotypes were derived from direct genotyping. Mann-Whitney tests were used to estimate differences in age at operation. The *P* values are two-sided. Hazard ratios were calculated using the Cox proportional hazard model, adjusting for gender and year of birth. The box-plots in a), c), and e) show the age at THR operation by genotype; the bottom and top of the boxes correspond to the 25^{th} (Q1) and 75^{th} (Q3) percentiles, the line inside the box the median age and the whiskers are located at Q1 - 1.5 IQR and Q3 + 1.5 IQR, respectively (where IQR is the interquartile range = Q3 - Q1).

Figure 2. Regional association plot for the 22q13.2 *CHADL* locus.

a) *P*-values (−log10) of variant association with THR under a recessive model in the Icelandic samples are plotted against their NCBI Build 38 positions at the 22q13.2 locus as black circles. Red crosses represent association with THR after adjusting for the homozygous state for rs532464664 (variant indicated by a vertical broken line). Known genes in the region are shown underneath the plot, taken from the UCSC genes track in the UCSC Genome Browser.
b) A zoom-in on the *CHADL* gene (RefSeq accession: NM_138481).

Figure 3. *CHADL* gene expression in human joint tissues, primary cells and mesenchymal stem cell (MSC) differentiation.

a) *CHADL* gene expression in cartilage (Cart) from non-OA neck of femur (NOF) fractures (n = 7), in cartilage (n = 7) and trabecular bone (n = 6) from OA hips and in cartilage (n = 19), trabecular bone (n = 10), synovium (Syn; n = 10) and fat pad (FP; n = 9) from OA knees.
b) *CHADL* gene expression in cultured human articular chondrocytes (HACs; n = 7) and human osteoblasts (HOBs; n = 4) from OA knees.
Data is expressed as mean ± standard error of the mean. *P*-values are two-sided.

c) *CHADL* gene expression in undifferentiated healthy MSCs and after 14 days of differentiation into chondrocytes (Chondro), adipocytes (Adipo) and osteoblasts (Osteo).

Table 1. Association of c.1141G>C in *COMP* (0.026%) and homozygous state of rs532464664 (0.15%) in *CHADL* with osteoarthritis in Iceland

		c.1141G>C in *COMP* (multiplicative model)[a]			rs532464664 in *CHADL* (recessive model)[b]		
Phenotype	**N_{cases} / $N_{controls}$**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Total hip replacement	4,657 / 207,514	16.7	7.5, 36.9	4.0×10^{-12}	7.71	4.86, 12.25	4.5×10^{-18}
Total knee replacement	3,289 / 170,001	1.14	0.24, 5.50	0.87	2.35	1.17, 4.72	0.016
Hand osteoarthritis	8,514 / 235,277	3.48	1.49, 8.11	0.0039	1.81	1.08, 3.03	0.023

Phenotypes of associations are shown with the number of cases and control.

[a] Association results under a multiplicative model for the missense variant c.1141G>C in the *COMP* gene (hg38 genomic position chr19:18,787,521, NM_000095.2:c.1141G>C, NP_000086.2:p.Asp369His). The frequency of the g.chr19:18,787,521[G] allele is 0.026% in Iceland. The odds-ratio is given with respect to the minor allele G of the variant. *P* values are two-sided.

[b] Association results under a recessive model for the 8 bp insertion allele CGCGCGCC at hg38 position chr22:41,238,083 in exon 3 of the *CHADL* gene (NM_138481.1:c.988_989insGGCGCGCG, NP_612490.1:p.Val330GlyfsTer106). The 8 bp insertion allele has 3.92% allele frequency in Iceland. The odds-ratio is given for the homozygous genotype of the minor allele insCGCGCGCC. *P* values are two-sided.

Online Methods

STUDY POPULATIONS

Iceland: The information on total hip replacements comes from a national Icelandic hip or knee arthroplasty registry[20,21] and from Landspitali University Hospital electronic health records. The registry was generated with computer-aided search of hospital records on all patients who had undergone total joint replacements for primary osteoarthritis of the hip or knee from any of the 6 orthopedic clinics in Iceland during the period from 1972 up to, and including, 2008. A clinician reviewed all patient records in 3 of the 6 hospitals and determined that the proportion of correct diagnosis was greater than 98%. Additionally, computer-aided search of joint replacement operations was performed in the Landspitali University Hospital's electronic health records up to, and including, 2015. Diagnoses such as hip fracture, rheumatoid arthritis and secondary causes of osteoarthritis, such as Perthes disease, hip dysplasia and slipped epiphysiolysis, were excluded. The total knee replacements were drawn from the same registry and from the Landspitali University Hospital. Those who had ruptured their anterior crucial ligament were also excluded from the knee replacements list. Because not all patient records could be reviewed for confirmation of diagnosis, we only included patients 40 years and older at the time of hip replacement in the genome-wide association scan. However, for the analysis of age at hip replacement and the survival analysis we also included individuals who had surgery at an earlier age. The average age, height and weight by gender of those undergoing THR and controls are shown in **Supplementary Table 10**.

Hand osteoarthritis patients were drawn from a database of 8,500 hand osteoarthritis patients that was initiated in 1972[22]. Participants were considered to have hand osteoarthritis if they had either definite finger osteoarthritis or definite thumb osteoarthritis, or both. Finger osteoarthritis was defined as definite bony swellings on at least one distal interphalangeal joint on each hand. Thumb osteoarthritis was defined as having definite squaring and/or dislocation of the first carpometacarpal joint.

Information on radiographic hip osteoarthritis was drawn from the Landspitali University Hospital, the total joint replacement registry and the hand osteoarthritis database.

All participants gave informed consent and the study was approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland.

Other populations: The Swedish samples were from the Swedish Malmo Diet and Cancer (MDC) study of men and women living in the city of Malmo in Sweden who were born between 1923 and 1945 (men) or between 1923 and 1950 (women). The Danish samples are postmenopausal women in the age range 55–86 years, taking part in the Prospective Epidemiological Risk Factor (PERF study)[23]. The Norwegian samples consisted of patients from memory clinics with cognitive symptoms or dementia and controls recruited from orthopaedic clinics, all enrolled in the DemGene project[24]. The Dutch study subjects are from the "Nijmegen Biomedical Study"[25]. The samples from the UK included individuals visiting the Leicester Royal Infirmary, Leicester and the Royal Hallamshire Hospital, Sheffield, and individuals from three primary care practices located in the same geographical area[26], and the arcOGEN sample collection of individuals who had undergone total joint replacement at nine different centers in the UK[8]. The Spanish individuals are part of a larger collection of control samples of European descent obtained from individuals who had attended the University Hospital in Zaragoza, Spain between November 2001 and May 2007. All controls are of self-reported European descent[27]. The samples from USA are from subjects taking part in a study of Polycystic Ovary Syndrome at Massachusetts General Hospital, Boston, USA[28]. Only individuals of European descent were included in this study. The Iranian samples are part of the Tehran Lipid and Glucose Study (TLGS) cohort[29]. The Chinese Hong Kong samples are comprised of two samples of different sex, the Mr OS and Ms OS studies, aged 65 years and above[30]. The Korean samples are postmenopausal women who visited the Osteoporosis Clinic of Asan Medical Center (AMC, Seoul, Korea)[31]. All participants in these studies provided informed consent and we obtained approval from all institutional review board to carry out the study.

GENOTYPING, IMPUTATION AND ASSOCIATION ANALYSIS: Genotyping and imputation methods and the association analysis method in the Icelandic samples were essentially as

previously described[32] with some modifications that are described here. In short, we sequenced the whole genomes of 8,453 Icelanders using Illumina technology to a mean depth of at least 10X (median 32X) (**Supplementary Fig. 16**). The sequencing was performed using the following three different library preparation methods and sequencing instruments from Illumina: (i) standard TruSeq DNA library preparation method; Illumina GAIIx and/or HiSeq 2000 sequencers; (ii) TruSeq DNA PCR-free library preparation method; Illumina HiSeq 2500 sequencers; and (iii) TruSeq Nano DNA library preparation method; Illumina HiSeq X sequencers (see **Supplementary Note** for a detailed description of the sequencing methods). SNPs and indels were identified and their genotypes called using joint calling with the Genome Analysis Toolkit HaplotypeCaller (GATK version 3.3.0)[33]. Genotype calls were improved by using information about haplotype sharing, taking advantage of the fact that all the sequenced individuals had also been chip-typed and long range phased. The sequence variants identified in the 8,453 sequenced Icelanders were then imputed into 150,656 Icelanders who had been genotyped with various Illumina SNP chips and their genotypes phased using long-range phasing[34,35]. Using genealogic information, the sequence variants were imputed into 294,212 un-typed relatives of the chip-typed to further increase the sample size for association analysis and increased the power to detect associations. Individuals who have undergone total hip replacements and controls were either chip-typed individuals ($N_{affected}$ = 3,110, $N_{controls}$ = 99,911) or first/second degree relatives of chip-typed individuals that were not chip-typed themselves ($N_{affected}$=1,547, $N_{controls}$=107,603). The number of affected males was 2,163 and affected females was 2,494. Association testing for case-control analysis was performed using logistic regression, adjusting for gender, age and county. A total of 31.6 million variants were used in the association analysis under a multiplicative model. For the recessive analysis, the number of tested variants for which we had homozygotes for the minor allele was 19.2 million. All of the variants that were tested had imputation information over 0.8.

To account for relatedness and stratification within the case and control sample sets, we applied the method of genomic control[36]. The inflation factor λ_g in the χ^2 statistic in each genome-wide analysis was estimated on the basis of a subset of about 300,000 common variants, and *P*-values were adjusted by dividing the corresponding χ^2 values by this factor. For the traits reported

here, the estimated inflation factors for the multiplicative (recessive) models were 1.29 (1.11) for total hip replacement, 1.16 (1.06) for total knee replacement, 1.32 (1.13) for hand OA and 1.04 (1.00) for radiographic hip OA without hip replacement.

We used the weighted Holm-Bonferroni method[37] to allocate familywise error rate of 0.05 equally between three annotation-based classes of sequence variants [9]. For the multiplicative model, this yielded significance thresholds of 1.7×10^{-6} for loss-of-function (LoF) variants (including stop-gained, frameshift, splice acceptor or donor; N=9,989), 9.8×10^{-8} for missense, splice-region variants and in-frame indels (N=170,692), and 5.3×10^{-10} for other (non-coding) variants (N=31,421,778); the significance thresholds and number of variants tested under the recessive model were 2.5×10^{-6} (N=6,799), 1.3×10^{-7} (N=124,475) and 8.7×10^{-10} (N=19,083,372), respectively.

SINGLE VARIANT GENOTYPING: The c.1141G>C (g.chr19:18787521) variant in *COMP* was genotyped using the Centaurus (Nanogen) platform[38]. The 8 bp *CHADL* frameshift insert was genotyped using a PCR based method with the primers shown in **Supplementary Table 11**. An internal size standard was added to the resulting PCR products and the fragments were separated on an Applied Biosystems model 3730 sequencer and then detected and called using in-house Allele Caller software.

RNA SEQUENCING ANALYSES:

Preparation of Poly-A cDNA sequencing libraries. Total RNA was isolated from PaxGene (Qiagen) blood tubes. The quality and quantity of isolated total RNA samples was assessed using the Total RNA 6000 Nano chip for the Agilent 2100 Bioanalyzer. cDNA libraries derived from Poly-A mRNA were generated using Illumina‘s TruSeq RNA Sample Prep Kit. Briefly, Poly-A mRNA was isolated from total RNA samples (1–4 μg input) using hybridizaton to Poly-T beads. The Poly-A mRNA was fragmented at 94°C, and first-strand cDNA was prepared using random hexamers and the SuperScript II reverse transcriptase (Invitrogen). Following second-strand cDNA synthesis, end repair, addition of a single A base, adaptor ligation, AMPure bead purification,

and PCR amplification; the resulting cDNA was measured on a Bioanalyzer using the DNA 1000 Lab Chip.

Sequencing. Samples were clustered on to flowcells using Illumina's cBot and the TruSeq PE cluster kits v2. Paired-end sequencing was performed with either GAIIx instruments using the TruSeq SBS kits v5 from Illumina or HiSeq 2000 instruments using TruSeq v3 flowcells/SBS kits; read lengths were 2×125 cycles.

Read alignment. RNA sequencing reads were aligned to Homo sapiens Build 38 with TopHat version 2.0.12 with a supplied set of known transcripts in GTF format (RefSeq hg38; Homo sapiens, NCBI,build 38). TopHat was configured such that it attempts first to align reads to the provided transcriptome then, for reads that do not map fully to the transcriptome, it attempts to map them onto the genome. Unmapped reads and reads aligning within 20 kb flanking region of *CHADL* were extracted and re-aligned using the Burrows-Wheeler Aligner (BWA-MEM 0.7.10) to the *CHADL* RefSeq transcript NM_138481.1 with and without the 8 bp insertion (HGVSc for mutated transcript: NM_138481.1:c.981_988dupGGCGCGCG); low quality reads were filtered out with Sickle 35 (version 1.33) prior to realignment and the minimum alignment score 80 was used in BWA-MEM.

RNA expression. TopHat aligned reads were used for quantifying *CHADL* expression for association testing for rs532464664. HTSeq-count[39] was used to count fragments aligning to genes. Count values were normalized with Trimmed Mean of M-values[40] method implemented with edgeR (v. 3.12.1) Bioconductor package.

Expression association. Linear regression assuming additive genetic effect was performed on log-transformed RNA expression estimates. The following covariates were used to correct for technical differences in the RNA experiments: average fragment length, exonic rate, number of genes detected, number of mapped read pairs, number of alternative alignments and percentage of reads originating from coding bases.

QUANTITATIVE PCR TISSUE PANEL EXPRESSION ANALYSES:

cDNA was synthesized using the High capacity cDNA reverse transcriptase kit (Applied Biosystems Inc.) on total RNA from several tissues (Cartilage tissue from DV biologics cat# SKU: AM009-R and the rest from Clonetech). Expression assays for the *CHADL* gene were designed using Roche universal probe library (Prod#: 04683633001), one for exon 2-3 junction and another for exon 3-4 junction (**Supplementary Table 11**). Real-time PCR was carried out according to manufacturer's recommendations on an ABI Prism 7900HT Sequence Detection System. Human *GUSB* (Applied Biosystems) was used to test the quality of the cDNA and correct for slight variance in input amount. Reactions were performed in duplicate, representative results are shown in **Supplementary Fig. 14**.

QUANTITATIVE PCR EXPRESSION ANALYSES OF JOINT TISSUES ISOLATED FROM TOTAL JOINT REPLACEMENTS:

Patients and samples: Joint tissue samples were obtained from patients with primary osteoarthritis (OA) or with neck of femur (NOF) fracture undergoing total knee (OA patients) or total hip (OA and NOF patients) replacement surgery at the Newcastle upon Tyne NHS Foundation Trust hospitals. The Newcastle and North Tyneside Research Ethics Committee granted ethical approval for the collection of the samples (REC reference number 14/NE/1212), with each donor providing informed consent. NOF patients showed no signs or symptoms of hip OA, and their cartilage was macroscopically intact and with no lesions.

Samples were stored at 4^{o}C in Hank's Balanced Salt Solution (HBSS) supplemented with Penicillin-Streptomycin and Nyastatin. Articular cartilage (distal from the lesion for the OA patients), trabecular bone, synovium and fat pad were obtained from the different patients when available.

The tissue samples that were to be used for RNA extraction were snap-frozen after collection, with the fresh trabecular bone samples being chopped in TRIzol® reagent (Ambion). These samples were then stored at -80^{o}C.

Cell culture: Cartilage from OA knees was processed for human articular chondrocyte (HAC) isolation. Cartilage was digested with 1 mg/ml Hyaluronidase at 37^{o}C for 15 min followed by 2.5 mg/ml trypsin at 37^{o}C for 30 min. Then, cartilage was incubated with 2.5 mg/ml collagenase at 35.5^{o}C overnight. Isolated chondrocytes were plated and cultured in DMEM containing 10% FBS and Penicillin-Streptomycin for 10 days.

Human osteoblasts (HOB) were obtained by culturing bone pieces from OA knees in culture dishes with DMEM containing 20% heat-inactivated FBS and Penicillin-Streptomycin until the cells reached confluence.

Human mesenchymal stem cells (MSCs) from a healthy donor were purchased from Lonza, UK. MSCs were differentiated into chondrocytes for 14 days as previously described [41]. For osteogenic differentiation, cells were seeded at 1.3 x 10^4/cm^2 and cultured in DMEM containing 10% FBS, Penicillin-Streptomycin, 0.1 µM dexamethasone, 10 mM β-glycerophosphate and 50 µM ascorbic acid for 14 days. Adipogenesis was achieved by two cycles of adipogenic induction for 3 days (DMEM supplemented with 10% FBS, 1 µM dexamethasone, 10 µg/ml insulin, 0.5 mM isobutylmethylxanthine, 60 µM indomethacin, 2 µM Rosiglitazone and 20 nM IGF-1) followed by 4 days in maintenance medium (DMEM supplemented with 10% FBS and 10 µg/ml insulin).

RNA isolation and cDNA synthesis: RNA from synovium, fat pad and cultured cells was isolated using E.Z.N.A.® DNA/RNA Isolation Kit (Omega Bio-tek) following manufacturer's protocol.

Trabecular bone RNA was isolated using TRIzol® reagent according to manufacturer's protocol, and RNA from cartilage was extracted using TRIzol® reagent followed by RNeasy Mini Kit (Qiagen) as previously described [42].

1 µg of RNA was used for cDNA synthesis using SuperScript™ First-Strand Synthesis System (Invitrogen) after digestion at 37^{o}C for 30 min with TURBO™ DNase (Ambion), following manufacturer's protocol.

Gene expression analysis by quantitative real-time PCR: Gene expression was determined using an ABI PRISM 7900HT Fast Real Time PCR System (Applied Biosystems). All reactions were performed in triplicate, and *CHADL* mRNA levels were normalised to *GAPDH*, *HPRT1* and *18S* and expressed using $2^{-\Delta Ct}$. Primers and probes used are given in **Supplementary Table 11**.

Statistical analysis was performed using Kruskal-Wallis test, followed by Dunns post test, or Mann-Whitney test when appropriate. All analyses were performed using Prism v6 software (GraphPad Software Inc). *P* (two sided) < 0.05 was considered statistically significant.

NONSENSE MEDIATED DECAY REPORTER ASSAY

Construction of NMD reporter system. The *HBB* and *CHADL* gene fragments were amplified using Phusion High-Fidelity polymerase (NEB) from human genomic DNA derived from human peripheral blood. CHADL gene fragments were obtained from a carrier and non-carrier of the 8 bp insert, whereas a premature translation termination codon was introduced at the start of the sequence of HBB using the HBB_R_STOP that created a target for NMD. The gene fragments correspond to chr11:5,246,722-5,248,029 (UCSC Genome Browser on Human Feb. 2009 GRCh37/hg19 Assembly) and chr22:41,632,489-41,634,216 (UCSC Genome Browser on Human Feb. 2009 GRCh37/hg19 Assembly) for HBB and CHADL respectively. The primers used for PCR are given in **Supplementary Table 11**. All sequences were verified by Sanger sequencing using standard methodology. The pmirGLO (Promega E133A) vector was used as a backbone for the NMD reporters. In order to create fused gene sequences, the stop codon of the luc2 was removed by using Q5 Site-directed mutagenesis kit (NEB E0554S) with primers show in **Supplementary Table 11**. The sequence of the plasmid was verified by Sanger sequencing. Prior to cloning of gene constructs the vector was linearized with the NheI restriction enzyme (NEB R0131S). All gene fragments were cloned downstream of the luc2 with a Gibson cloning kit (NEB M5510A) following manufacturers recommendations creating a fusion protein of luc2 and the relevant gene fragments. Plasmids were isolated with QIAprep Spin miniprep kit (QIAGEN) and inserts were verified by Sanger sequencing.

Luminescence analysis. Two days prior to transfection, 30,000 HeLa cells (Public Health England 93021013) were seeded into each well of a 96-well plate in 200µL of DMEM medium (11995-065, ThermoFisher) supplemented with 10% fetal calf serum (ThermoFisher 10500-064) and 50 units/mL penicillin and 50 ug/mL streptomycin (ThermoFisher 15070-063). Cells were incubated overnight at 37°C and 5% CO2 in a humidified incubator. Each well was washed once

with PBS and 90µL of medium was added to each well without antibiotics and cells were incubated overnight. For each well, 100ng of plasmid DNA was transfected using Lipofectamine 3000 (ThermoFisher L30000-008). 48 hours post transfection luminescence was measured using the Dual-Glo Luciferase system (Promega E220) on a Glomax Discover (Promega GM3000). Data were presented as the luc2/hRluc-neo ratio calculated for each well. For inhibitor experiments, cells were cultured as described and then treated with 10mM caffeine for the last 24 hours prior to luminescence measurements or the last 8 hours with 20µM wortmaninn.

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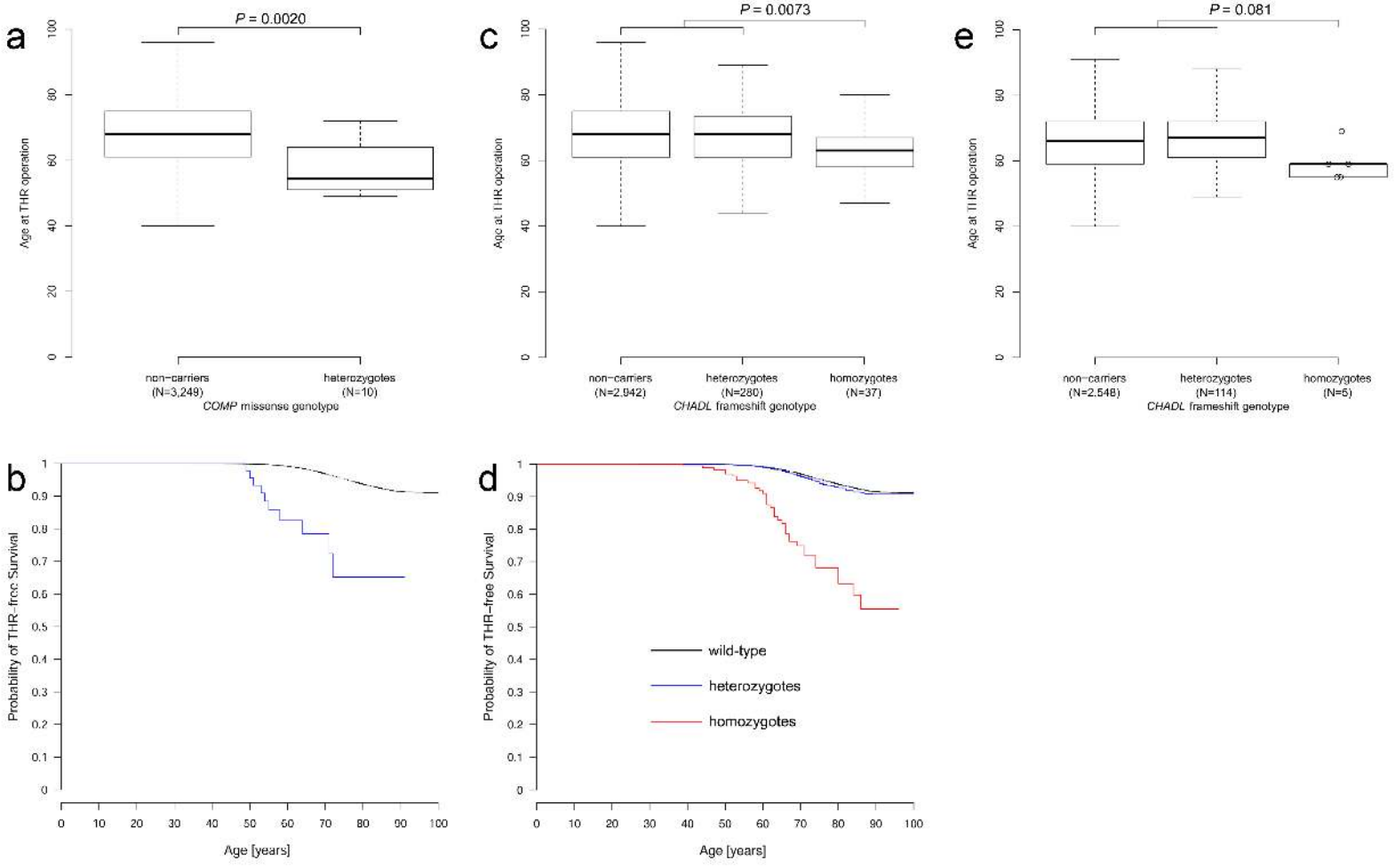
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Age at THR operation
non−carriers
(N=3,249)
heterozygotes
(N=10)
COMP missense genotype
c
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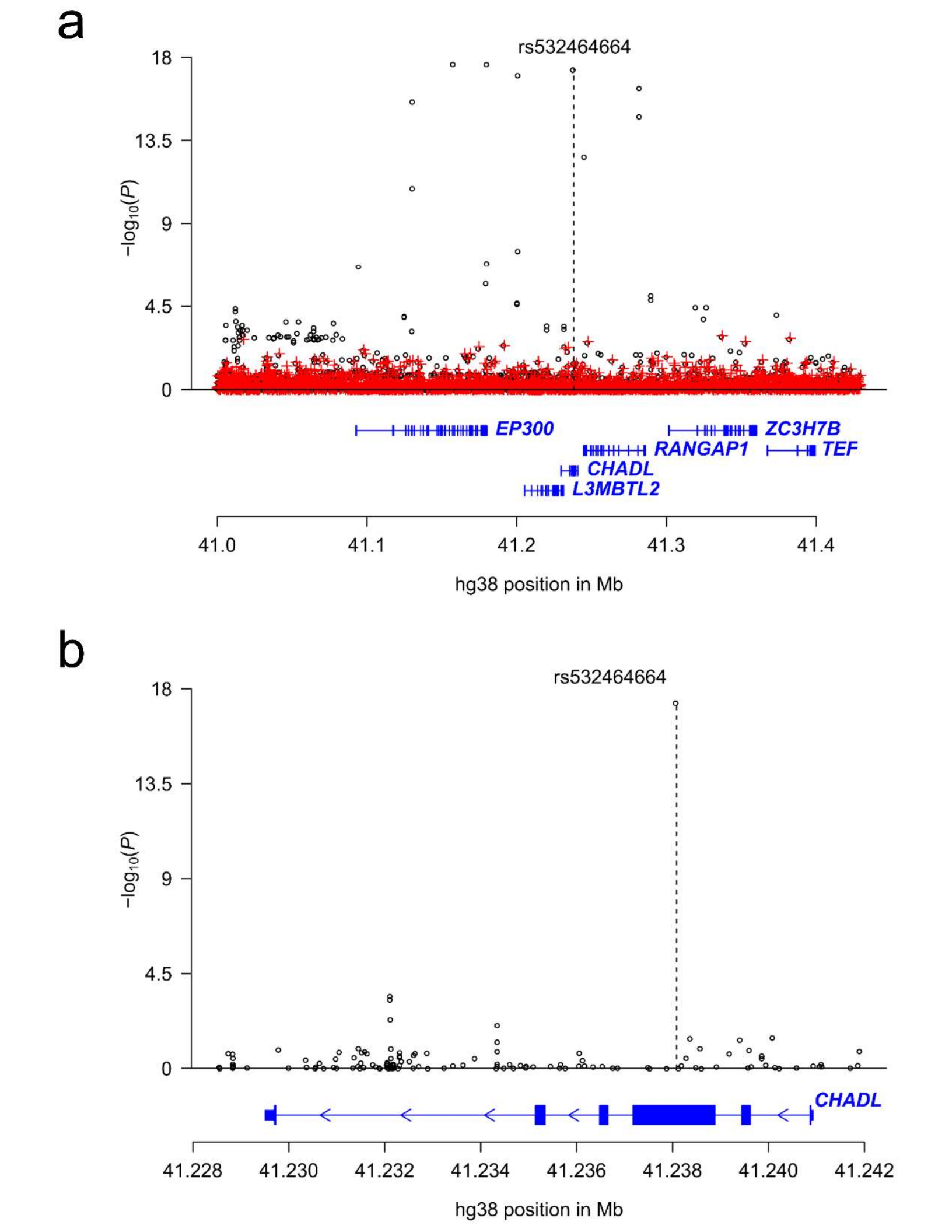
Age at THR operation
non−carriers
(N=2,942)
heterozygotes
(N=280)
homozygotes
(N=37)
CHADL frameshift genotype
e
P = 0.081
Age at THR operation
non−carriers
(N=2,548)
heterozygotes
(N=114)
homozygotes
(N=5)
CHADL frameshift genotype
b
Probability of THR−free Survival
Age [years]
d
Probability of THR−free Survival
wild−type
heterozygotes
homozygotes
Age [years]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CHADL
L3MBTL2
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41.1
41.2
41.3
41.4
hg38 position in Mb
b
rs532464664
18
13.5
9
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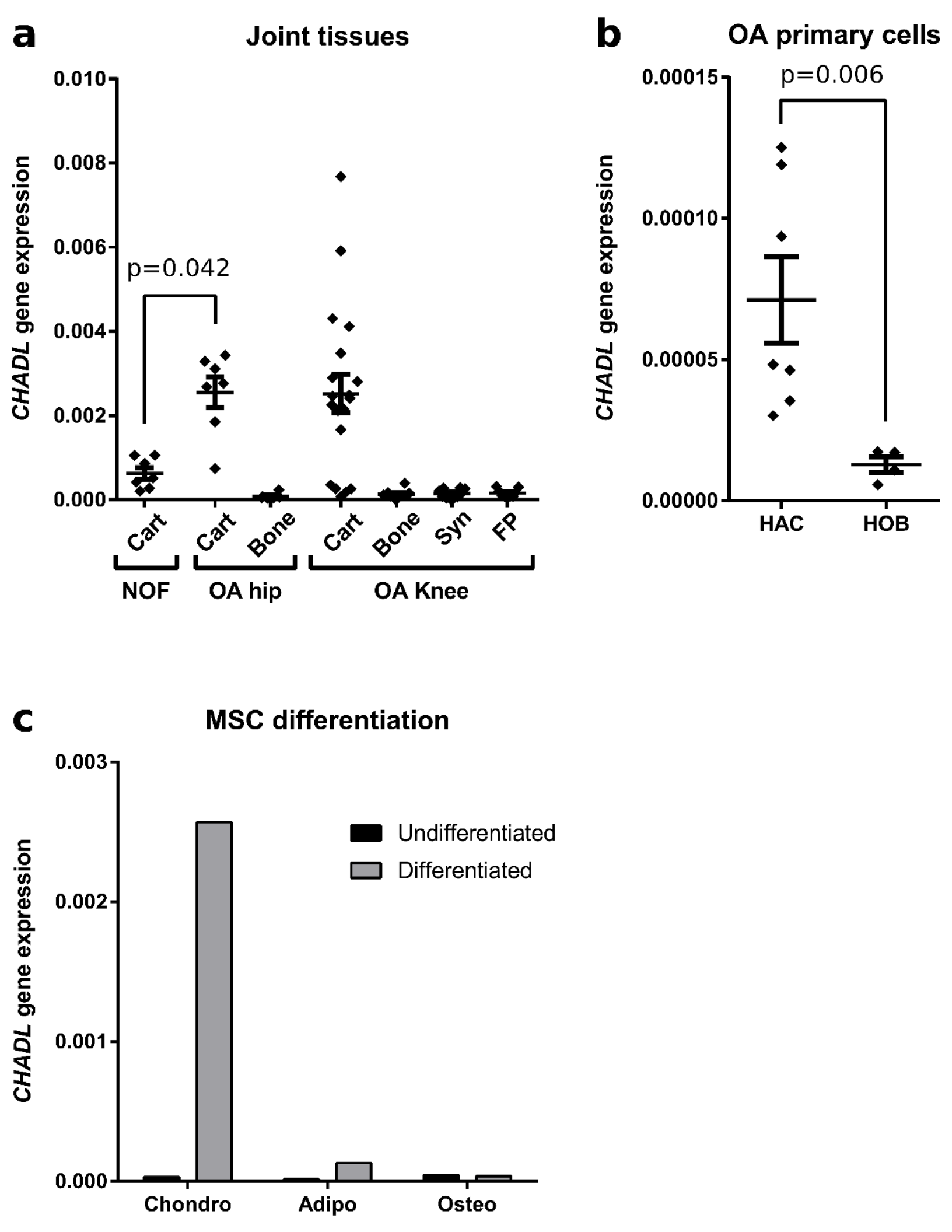
a
Joint tissues
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0.006
0.004
0.002
0.000
p=0.042
Cart
Cart
Bone
Cart
Bone
Syn
FP
NOF
OA hip
OA Knee
b
OA primary cells
CHADL gene expression
0.00015
0.00010
0.00005
0.00000
p=0.006
HAC
HOB
c
MSC differentiation
CHADL gene expression
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0.001
0.000
Undifferentiated
Differentiated
Chondro
Adipo
Osteo