

ARTICLE

Development of an Integrated Platform Using Multidisciplinary Real-World Data to Facilitate Biomarker Discovery for Medical Products

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Translational multidisciplinary research is important for the Center for Devices and Radiological Health's efforts for utilizing real-world data (RWD) to enhance predictive evaluation of medical device performance in patient subpopulations. As part of our efforts for developing new RWD-based evidentiary approaches, including *in silico* discovery of device-related risk predictors and biomarkers, this study aims to characterize the sex/race-related trends in hip replacement outcomes and identify corresponding candidate single nucleotide polymorphisms (SNPs). Adverse outcomes were assessed by deriving RWD from a retrospective analysis of hip replacement hospital discharge data from the National Inpatient Sample (NIS). Candidate SNPs were explored using pre-existing data from the Personalized Medicine Research Project (PMRP). High-Performance Integrated Virtual Environment was used for analyzing and visualizing putative associations between SNPs and adverse outcomes. Ingenuity Pathway Analysis (IPA) was used for exploring plausibility of the sex-related candidate SNPs and characterizing gene networks associated with the variants of interest. The NIS-based epidemiologic evidence showed that periprosthetic osteolysis (PO) was most prevalent among white men. The PMRP-based genetic evidence associated the PO-related male predominance with rs7121 (odds ratio = 4.89; 95% confidence interval = 1.41–17.05) and other candidate SNPs. SNP-based IPA analysis of the expected gene expression alterations and corresponding signaling pathways suggested possible role of sex-related metabolic factors in development of PO, which was substantiated by *ad hoc* epidemiologic analysis identifying the sex-related differences in metabolic comorbidities in men vs. women with hip replacement-related PO. Thus, our *in silico* study illustrates RWD-based evidentiary approaches that may facilitate cost/time-efficient discovery of biomarkers for informing use of medical products.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✔ Despite the widely recognized need for biomarkers and risk predictors for joint replacement-related adverse outcomes, there are presently no reliable study end points that can inform evaluation of real-world performance in patient subpopulations and enable pre-implantation testing for identifying individuals with enhanced susceptibility to implant-related adverse outcomes.

WHAT QUESTION DID THIS STUDY ADDRESS?

✔ This study addresses the role of sex in joint replacement-related outcome heterogeneity and identifies candidate single nucleotide polymorphisms (SNPs) for predicting the risk of periprosthetic osteolysis (PO) as a major adverse outcome with sexual dimorphism.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✔ This study elucidates the patient factors (sex and SNPs) underlying susceptibility to joint replacement-related adverse outcomes (PO). This study also illustrates the overall potential of *in silico* methodology for identifying biomarkers that are indicative of real-world performance and thereby can inform use of medical products and improve treatment outcomes.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✔ *In silico* biomarker discovery that reutilizes pre-existing genetic data and is guided by epidemiologic real-world evidence can pave the way for development of cost/time-efficient precision medicine applications.

Translational research is important for achieving the Center for Devices and Radiological Health (CDRH)'s goals and

priorities and providing access to safe and effective medical devices.¹ As part of the Center's regulatory research

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efforts to enhance predictive evaluation of real-world device performance, we are developing an *in silico* framework for integrating multidisciplinary (e.g., epidemiologic and genetic) real-world evidence (RWE) that can be used for identifying biomarkers and study end points indicative of device performance in different patient subpopulations.²

The current study is focused on new evidentiary approaches to evaluation of the hip replacement-related adverse outcomes. As a main treatment of end-stage osteoarthritis, hip replacement (also known as hip arthroplasty) has a staggering societal impact, which is projected to rise due to the growing aging population and, therefore, entails the demand for reliable long-term implant performance.^{3,4} Both periprosthetic osteolysis (PO) and resultant aseptic loosening are among the leading causes of implant failure and revision.⁵ Although the occurrence of PO seems to be decreasing with the advanced implant designs and increased knowledge about the implant's wear and biological responses to the resultant wear/corrosion debris, the continuing increase of arthroplasties necessitates better ways of managing PO and other implant-related adverse outcomes.⁶

Despite possible sex-related disparities in adverse outcomes pertaining to orthopedic implants⁷ and overall importance of demographics,⁸ its role in the implant-related outcome heterogeneity remains elusive due to the lack of studies reporting sex/race-stratified data. As a result, mechanistic understanding of patients' demographics as a risk modifier in implant-related outcomes is lacking. Although some studies addressed the obvious need for biomarkers predicting the risk of implant-related complications,^{9,10} none of the published studies, to our knowledge, provided comprehensive assessment of patient-related (genetic and nongenetic) factors that may impact adverse outcomes in hip replacement. To address the paucity of such studies, we attempted to characterize adverse outcomes in the sex/race-stratified hip replacement subpopulations and identify candidate single nucleotide polymorphisms (SNPs) that may predict the risk of adverse outcomes and indicate their molecular underpinnings in relation to demographic-related modifying effects.

In addition to discovery of actual candidate SNPs for PO, our study was focused on development of *in silico* methodology for reutilizing pre-existing real-world data (RWD) and integrating multidisciplinary—epidemiologic and genetic—evidence with healthcare applications. As a starting point, we analyzed putative sex/race-related trends associated with hip replacement. Following the derived epidemiologic RWE on higher risk of PO among white men, a subsequent biomarker discovery was focused on the sex/race-related biomarkers for PO and PO-related wear. *In silico* plausibility analysis of the candidate SNPs and corresponding gene expression correlations suggested that PO risk may be modified by the sex-related metabolic factors. Our *ad hoc* epidemiologic queries, which were prompted by the SNP-based evidence, further indicated the role of sex-related metabolic modification of PO risk. Thus, our study suggested the sex-related modifying effects on PO risk and subsequently identified candidate SNPs, which seem to be associated with the predominance of PO in white men. Most importantly, our study illustrated transferable approaches to *in silico* discovery of biomarkers for informing the use of medical products.

METHODS

Epidemiologic RWE on hip replacement outcomes was derived from a retrospective analysis using RWD from the National (Nationwide) Inpatient Sample (NIS; 2006–2012) from the Agency for Healthcare Research and Quality (AHRQ; a healthcare database accessible at <https://www.hcup-us.ahrq.gov/>) and the Personalized Medicine Research Project (PMRP)¹¹ of Marshfield Clinic Research Institute (MCRI). Joint replacement-related procedures and diagnoses were identified using the following International Classification of Diseases, Clinical Modification (ICD-9-CM) codes:

- V43.64: Hip joint replacement
- 81.51: Total hip replacement
- 81.52: Partial hip replacement
- 81.53: Revision of hip replacement not otherwise specified
- 00.70: Revision of hip replacement, both acetabular and femoral components
- 00.71: Revision of hip replacement, acetabular component
- 00.72: Revision of hip replacement, femoral component
- 00.73: Revision of hip replacement, acetabular liner, and/or femoral head only
- 996.41: Mechanical loosening of prosthetic joint
- 996.42: Dislocation of prosthetic joint
- 996.43: Broken prosthetic joint implant
- 996.44: Periprosthetic fracture around prosthetic joint
- 996.45: Periprosthetic osteolysis
- 996.46: Articular bearing surface wear of prosthetic joint

Adverse outcomes identified by 996.41–996.46 ICD-9-CM codes were primary outcomes of interest. Sex and race were the main covariates of interest. Total NIS records with hip replacement comprised 615,251 discharges. Hip replacement discharges were first encompassed by using V43.64. Discharge records with V43.64 but without 996.41–996.46 codes were considered as hip replacement with no adverse outcomes. Discharge records with V43.64 in combination with any of 996.41–996.46 codes were considered as hip replacement complicated by one or more adverse outcomes (e.g., V43.64 + 996.45 + 996.46 as discharges reflecting hip replacement complicated by PO and wear). Cases with revised hip replacement were identified by code combinations for both a revision procedure and an adverse outcome as possible underlying causes (e.g., 00.70 + 996.45 as hip replacement subpopulation with total revision due to PO). Additional ICD-9-CM and Clinical Classifications Software (CCS) codes for diabetes, obesity, and lipid disorders were used for epidemiologic analysis on metabolic comorbidities (more details can be found in the corresponding tables).

STATA 14 (Stata Corp, College Station, TX) was applied for comparative analysis of the frequencies of adverse outcomes in sex/race-stratified NIS discharges, using a custom-written algorithm adapted to filter NIS discharges with relevant ICD-9 codes. Frequency analysis, along with descriptive statistics, was performed using the two-tailed Student *t*-test for comparing continuous variables and the χ^2 test for categorical data; statistical significance was assessed at $P < 0.05$.

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to identify potential differences in the odds of adverse outcomes in different subgroups (e.g., white women vs. white men) and assess potential correlations between adverse outcomes and patient-related factors (e.g., sex/race and SNP alleles).

Cytel Studio software was used for statistical assessment of the main candidate SNP (rs7121); sensitivity, specificity, and positive and negative likelihood ratios (PLR and NLR, respectively) were calculated per ratio of two independent binomial proportions.

The PMRP¹¹ of the MCRI provided a cohort of patients with hip replacements (total = 871 patients) with pre-existing epidemiologic (demographics and ICD-9 codes, as shown above) and genetic (a total of 34 disease-related SNPs; **Table S1**) data for genotype-phenotype analysis. High-Performance Integrated Virtual Environment (HIVE), an in-house computing platform available at the US Food and Drug Administration (FDA; public HIVE domain at the George Washington University is accessible at <https://hive.biochemistry.gwu.edu/dna.cgi?cmd=main>), was used for hierarchical clustering, which was applied for analyzing and visualizing the sex-stratified associations between SNP alleles and adverse outcomes in hip replacements (additional details are provided in the legends of corresponding figures).

Open-access National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov>) databases, such as Gene and Gene Expression Omnibus, were used to explore SNPs of interest and corresponding genes. Open-access genomic browser Ensembl (<https://www.ensembl.org>) and Genotype-Tissue Expression portal data were used for characterizing SNPs, identifying expression quantitative trait loci (eQTLs), and corresponding gene expression alterations associated with the variants of interest. The population genetics data from 1000 Genomes accessible through Ensembl were used for comparing baseline SNP allele frequencies in different racial subgroups. The biomedical knowledge bases and causal analytics tools from Correlation Engine/BaseSpace (Illumina) and Ingenuity Pathway Analysis (IPA; Qiagen Bioinformatics) were used to explore possible gene/protein interactions, signaling/metabolic pathways, and upstream/downstream effects pertaining to candidate SNPs and corresponding eQTLs/genes. More details on *in silico* data sources

and methods used are provided in the legends of corresponding figures.

RESULTS

Epidemiologic evidence on sex/race-related trends pertaining to adverse outcomes in hip replacement

Based on the analysis of annual hospital discharges with V43.64 as the main procedure of interest (NIS, 2006–2012), women with hip joint replacement consistently outnumbered their male counterparts (as shown by the yearly discharge numbers in **Figure S1**). Subsequently, almost all adverse outcomes, including the most frequent mechanical loosening and dislocation, showed significant female predominance in both white and black subpopulations (**Figure S2**). The single adverse outcome that did not follow this pattern was PO, which was more frequent in black women ($P = 0.0017$), but not white women ($P = 0.1192$; **Figure 1**). A more detailed analysis in sex/race-stratified subpopulations (**Table 1**; more detailed demographic characterization can be found in **Table S2**) confirmed a substantially lower risk of PO in white women compared with white men (OR = 0.54; 95% CI = 0.50–0.58), whereas the same trend in the black subpopulation failed to reach significance levels. Women, however, were more likely to have dislocation and periprosthetic fracture.

Suggesting their causative role in device failure, almost all adverse outcomes (except broken implant) were more frequent among discharges with revision. Dislocation and periprosthetic fracture among white women with revision of both acetabular and femoral components increased by fourfold to fivefold compared with their counterparts with unrevised hip replacement (from 4.3% to 22.9% and from 1.4% to 5.4%, respectively). Sex/race-related modification of the revision-associated adverse outcomes was shown by ORs comparing the highest and the lowest frequencies for each outcome in white and black subpopulations (**Figure 2**). Dislocation and mechanical loosening remained the most frequent adverse outcomes among the revised hip replacements. Similar to their counterparts with no revision, the revision-associated dislocation and periprosthetic fracture maintained predominance of white women (OR_{WFvsBM} = 2.04; 95% CI = 1.76–2.34 and OR_{WFvsBM} = 1.68; 95% CI = 1.31–2.17, respectively).

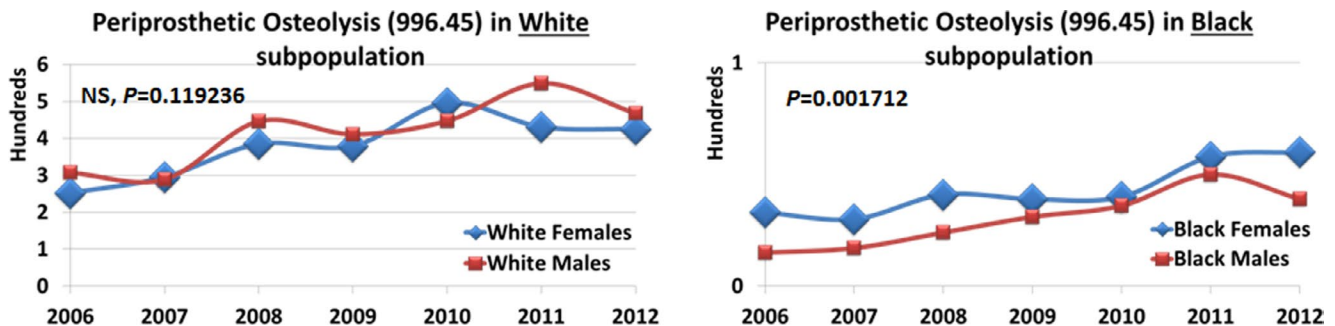


Figure 1 Sex/race-related trends in periprosthetic osteolysis, per annual discharge numbers (National Inpatient Sample/Agency for Healthcare Research and Quality (NIS/AHRQ)). The curves representing annual discharges in sex-stratified subpopulations are derived from STATA analysis of NIS/AHRQ discharges (2006–2012) pertaining to joint prostheses (including but not limited to hip replacement); P values are derived using two-tailed t -test ($P < 0.05$). NS, not significant.

Table 1 Sex/race-related trends in adverse outcomes pertaining to hip replacement

	ORs for adverse outcomes in sex/race-stratified subpopulations with hip replacement (OR [95% CI])					
	Mechanical loosening	Dislocation	Broken implant	Wear	Periprosthetic fracture	Periprosthetic osteolysis
All women vs. all men (<i>n</i> = 380,601 and <i>n</i> = 234,650, respectively)	0.80 [0.77–0.83]	1.17 [1.14–1.20]	0.85 [0.80–0.90]	0.71 [0.68–0.76]	1.31 [1.25–1.37]	0.56 [0.53–0.60]
White women vs. white men (<i>n</i> = 280,046 and <i>n</i> = 171,815, respectively)	0.80 [0.77–0.84]	1.16 [1.12–1.20]	0.86 [0.80–0.92]	0.70 [0.65–0.76]	1.32 [1.25–1.40]	0.54 [0.50–0.58]
Black women vs. black men (<i>n</i> = 25,833 and <i>n</i> = 17,524, respectively)	0.85 [0.75–0.98]	1.12 [0.99–1.26]	0.78* [0.61–1.00]	0.96* [0.71–1.30]	1.20* [0.94–1.52]	0.85* [0.67–1.08]

The presented trends are based on National Inpatient Sample/Agency for Healthcare Research and Quality discharges with hip replacement. “All women vs. all men” category includes all available race/ethnicity subcategories, some of which are not shown individually due to mostly nonsignificant ORs. ORs representing the most pronounced changes are highlighted in bold; 95% CIs including 1 are designated by an asterisk. This table represents numbers of discharge records (not numbers of patients).
CI, confidence interval; OR, odds ratio.

The revision-associated mechanical loosening was more frequent among black men ($OR_{BMvsWF} = 1.40$; 95% CI = 1.24–1.58). Although aseptic loosening is considered the end point of PO,⁵ the ICD-diagnosed PO showed a lower frequency compared with mechanical loosening, which may include other types of loosening. However, PO remained most frequent in white men who had revisions ($OR_{WMvsWF} = 1.64$; 95% CI = 1.53–1.77). Indicating possible associations with PO, wear was also more frequent in the white men with revisions ($OR_{WMvsBM} = 1.33$; 95% CI = 1.05–1.70).

Similar to the NIS-derived sex distribution, the PMRP subcohorts with hip replacement, especially with no adverse outcomes, showed female predominance, with the male predominance limited to the PMRP subcohort with PO (M/F ratio = 1.29).

Thus, higher risk of PO in white men represented the single trend that overcame overall female predominance in hip replacement.

Genetic evidence on candidate SNPs associated with adverse outcomes in the sex/race-stratified subpopulations with hip replacements

The presence of sex/race-related trends indicates possible genetic factors underlying adverse outcomes in hip replacements. Osteolysis and loosening were previously associated with SNPs affecting inflammatory pathways, bone turnover, and tissue remodeling.^{9,12–15} GNAS-rs7121 in particular was initially suggested as a risk factor for aseptic loosening in males.¹⁶ Although the subsequent evidence linking rs7121 to aseptic loosening seems contradictory,^{17,18} GNAS-rs7121, to our knowledge,

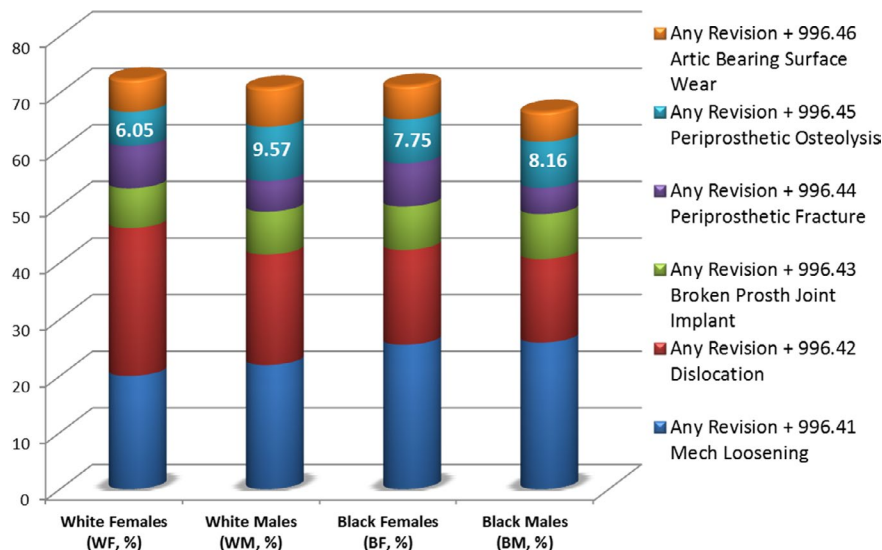


Figure 2 Periprosthetic osteolysis (PO) and other adverse outcomes in sex/race-stratified patient subpopulations with revised hip replacement. “Any Revision” category combines discharges with different revisions pertaining to hip replacement (International Classification of Disease 9th revision: 81.53; 00.70–00.73). Percentages of PO (6.05–9.57%, as shown) and other adverse outcomes (not shown) are based on denominators representing total numbers of subjects with revised hip replacements in each sex/race-stratified subgroup. The y-axis represents cumulative percentages of revision-related adverse outcomes in sex/race-stratified subgroups.

remains the only SNP that has been thus far associated with the sex-related differences in hip replacement outcomes. Because GNAS-rs7121 and some other SNPs linked to adverse outcomes in hip replacement were included into the panel of disease-associated SNPs used in the PMRP,^{11,19,20} we utilized pre-existing PMRP SNP data (**Table S1**) to test the putative sex-dependent role of GNAS-rs7121 and other available SNPs in the hip replacement-related adverse outcomes.

Similar to the original PMRP cohort,²⁰ the PMRP sub-cohort with hip replacement was predominantly white (97.4%), and GNAS-rs7121 genotypes were compatible with Hardy-Weinberg equilibrium in both men and women. However, the PMRP subcohort with hip replacement-related PO showed most of white men carrying rs7121-C allele (**Figure 3a**).

HIVE unsupervised clustering revealed a heatmap with the sex-specific subclusters presenting SNP associations with adverse outcomes (blue and pink rectangles for men and women, respectively), where PO was associated with different SNP alleles in men vs. women (**Figure 3b**). The increase of rs7121-C allele in white men with PO was accompanied by higher frequencies (as indicated by dark yellow squares) of additional four SNP alleles, none of which were found in the subcluster of white women with PO (which was characterized by lower frequencies of other SNP alleles, as indicated by dark blue squares).

As shown in **Figure 3c**, white men with GNAS_rs7121-C allele had a fivefold higher risk of hip replacement-related PO (OR = 4.89; 95% CI = 1.41–17.05), compared with control male subjects with no PO. The risk of PO among white men was also significantly associated with β 2-adrenergic receptor 2 (ADRB2)_rs1042714-G (OR = 3.28; 95% CI = 1.13–9.53).

Only two candidate SNPs were associated with the sex-independent risk for adverse outcomes. ADRB2_rs1042714-C was significantly decreased in subgroups with wear and PO (OR = 0.38; 95% CI = 0.20–0.71 and OR = 0.34; 95% CI = 0.16–0.73, respectively). MMP2_rs243865-C was borderline increased in the subgroup with PO (OR = 3.21; 95% CI = 0.97–10.58).

Our further statistical assessment focused on rs7121 as the main sex-associated candidate SNP. Sensitivity, or true-positive rate, of the rs7121-C allele among white men

with PO was assessed as 83.3% (15/18), whereas the corresponding false-positive rate among white men with no PO reached only 48.8% (164/336). Subsequently, PLR and NLR for the rs7121-C allele as a predictor of PO in white men were estimated as 1.71 and 0.33, respectively, suggesting a stronger risk modification by the absence rather than presence of the C-allele (i.e., 3-fold reduction vs. 1.7-fold increase, respectively). The subsequent dominant protective effect suggested for T-allele was consistent with the Ensembl-derived predictions, which limited rs7121 deleterious effects to non-T alleles. Further indicating the male sex-dependent risk modification, rs7121-C allele did not reach meaningful PLR and NLR values (0.77 and 1.30, respectively) as a predictor of PO among white women. As expected, PLR and NLR estimates for rs7121-C allele in cases with no PO remained close to unity.

IPA-based functional plausibility results on the PO-associated SNP candidates

Next, we applied IPA for exploring possible functional consequences from SNPs that correlated with PO and wear in men (**Figure 3c**). IPA knowledge base was queried to create the list of osteolysis-related genes (x43), which was then compared with our list of candidate SNP-harboring genes (x7). The rs243865-harboring MMP2 was the only gene shared by the IPA list of osteolysis-related genes (**Figure S3**), with rs243865 being implicated in bisphosphonate-induced osteonecrosis²¹ and other MMP2 SNPs—in multicentric osteolysis (SNPedia).

The rs7121-harboring GNAS locus (20q13.32) is known for its associations with bone diseases, including osteolytic changes (NCBI/Gene). According to our *in silico* research using NCBI and Correlation Engine databases, some GNAS SNPs were implicated in hip geometry (Framingham study) and GNAS gene expression was significantly higher in aseptic (wear particle-induced) vs. septic loosening (GSE7103 study accessible at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7103>, NCBI).

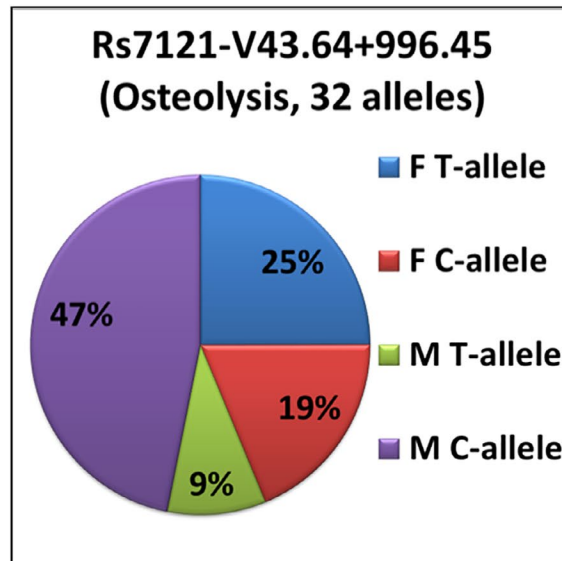
As a next step, we used Ensembl/Genotype-Tissue Expression portal data to derive the lists of eQTLs (**Table S3**) pertaining to candidate SNPs from male-PO and female-PO subclusters (as highlighted by blue and pink rectangles, respectively, in **Figure 3b**). IPA comparison of the eQTL-associated genes revealed two distinct top pathways:

Figure 3 Sex-related associations between single nucleotide polymorphisms (SNPs) and adverse outcomes in hip replacement. (a) GNAS_rs7121 allele distribution in periprosthetic osteolysis (PO) and other adverse outcomes in sex-stratified Marshfield Clinic Research Institute (MCRI) subgroups with hip replacement. Note: Some subjects with hip replacement had more than one adverse outcome (e.g., osteolysis and wear) and, therefore, were included into more than one MCRI subgroup. (b) High-Performance Integrated Virtual Environment (HIVE)-based clustering heatmap presenting correlations between adverse outcomes and SNP alleles in sex-stratified MCRI subgroups with hip replacement. The heatmap was created using HIVE unsupervised clustering algorithm. Rows show stratification by SNP alleles in male and female subgroups with certain SNP allele (e.g., M_7121_C refers to the C-allele of rs7121 in male patients). Columns show stratification by International Classification of Diseases revision 9 (ICD-9) codes. Figure legend represents a relative scale of allele frequencies: from 0 as lowest (dark blue) to 1 as highest (dark yellow). Two enlarged heatmap fragments represent the most noticeable allele changes in male and female subclusters (blue and pink boxes, respectively) with hip replacement-related PO (e.g., V43.64 + 996.45). (c) HIVE-based clustering heatmap fragments with the most significant correlations between PO or wear and certain SNP alleles in MCRI male subjects with hip replacement. The top section shows SNP alleles (x4) with highest frequencies (as designated by dark yellow) among men with PO. The bottom section represents SNP alleles (x3) with lowest frequencies (as designated by dark blue) among men with wear as well as a single SNP allele with highest frequency (as designated by dark yellow) among females with wear. Corresponding odds ratios (ORs) are shown on the left; solid and dash borders around the squares represent 95% confidence intervals (CIs) not including 1 and close to 1, respectively. F, female; M, male.

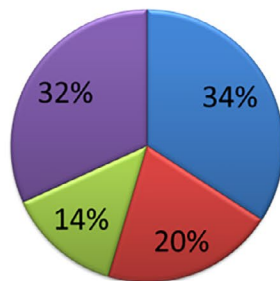
cAMP/G-protein-coupled receptor signaling in the male-PO and farnesoid X receptor/ retinoid X receptor activation in the female-PO (**Figure S4**), both of which are involved in metabolic regulation.

As the key element of cAMP/G-protein-mediated signal transduction, GNAS is involved in energy production

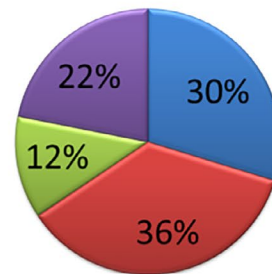
and lipid metabolism. Although the role of GNAS-rs7121 in obesity is not fully elucidated,²² rs7121-CC genotype was associated with obesity and insulin resistance.²³ The rs1042714-harboring ADRB2 is associated with regulation of energy expenditure and lipid mobilization, and the sex-dependent obesity associations of



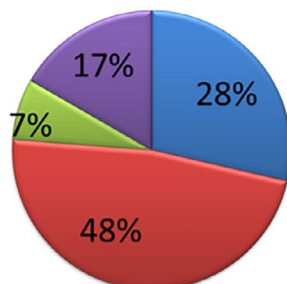
**Rs7121-V43.64+996.44
(Fracture, 44 alleles)**



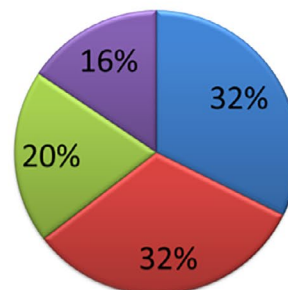
**Rs7121-V43.64+996.42
(Dislocation, 64 alleles)**



**Rs7121-V43.64+996.46
(Wear, 42 alleles)**



**Rs7121-V43.64+996.41
(Mech_Loos, 96 alleles)**



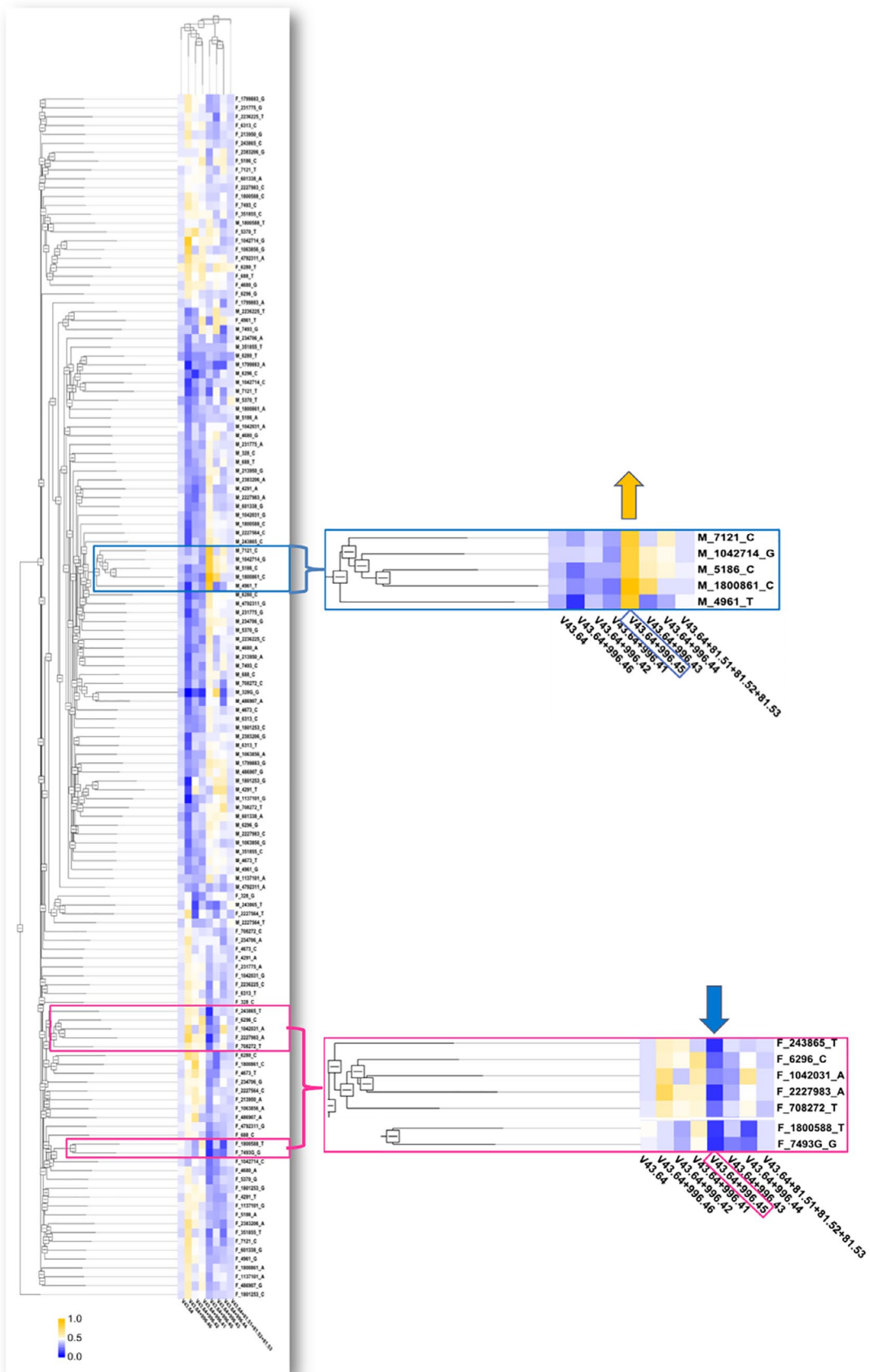


Figure 3 (Continued)

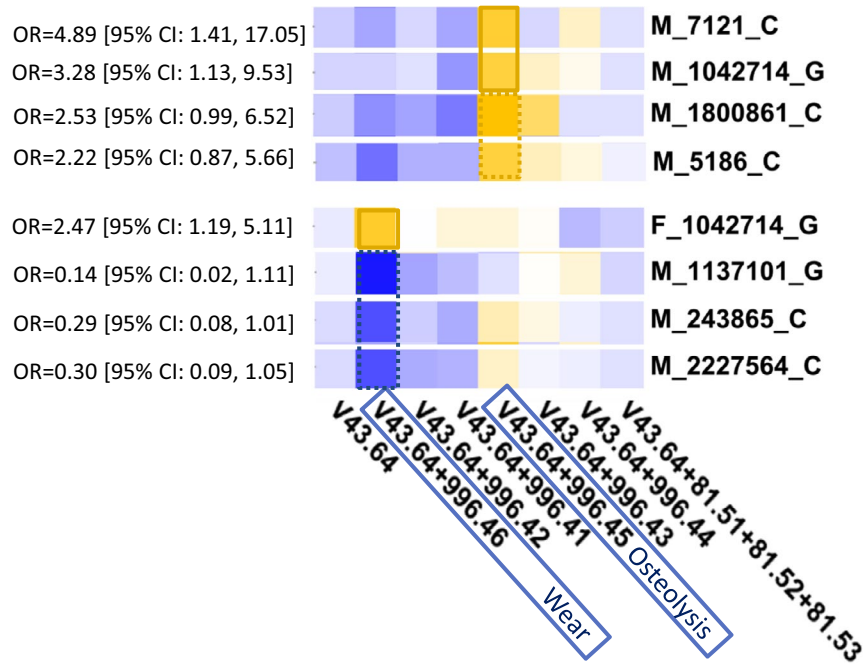


Figure 3 (Continued)

ADRB2_rs1042714 were shown to involve triglyceride levels in Asian men, but not women.^{24–26} The ADRB2_rs1042714-G allele, in particular, was decreased in obese men but increased in obese women.²⁷ In a study on non-diabetic Japanese subjects,²⁵ rs1042714 in ADRB2 and rs1137101 in leptin receptor (LEPR) conferred a synergistically increased obesity risk, especially in men. The LEPR_rs1137101_G allele was suggested to increase risk for diabetes,²⁸ whereas ADRB2_rs1042714_GG genotype was associated with a twofold reduced risk for diabetes.²⁹

Thus, the IPA-derived evidence on candidate SNPs and eQTLs from male-PO and female-PO clusters highlighted distinct mechanisms potentially responsible for the sex/metabolism-dependent modulation of PO in hip replacement.

Cross-substantiation of genetic and epidemiologic evidence on the sex-related risk of PO in hip replacement

To test the sex-related metabolic associations inferred by SNP-based genetic evidence, we conducted an *ad hoc* NIS-based epidemiologic analysis comparing the odds of developing PO among the sex-stratified hip replacement subpopulations with concurrent metabolic disorders. To match the white origin of PMRP subcohort, which provided candidate SNPs, this NIS data set was limited to hospital discharges with reported white race/ethnicity.

White women with obesity (CCS: 3.11.2) had a 24% increase in odds of having PO (Table 2; more detailed demographic characterization of subjects with metabolic disorders can be found in Table S4). Mixed hyperlipidemia (ICD-9: 272.2) increased PO risk in white women by almost

Table 2 Sex/race-related trends in PO pertaining to hip replacement

Metabolic comorbidities	ORs for PO among white subpopulation with hip replacement (OR [95% CI])		
	All	Women	Men
Disorders of lipid metabolism (single-level CCS 53, n = 297,273):	1.11 [1.02, 1.19]	0.98* [0.87, 1.10]	1.14 [1.03, 1.27]
Pure hypercholesterolemia (ICD-9 272.0, n = 92,898)	1.28 [1.14, 1.43]	1.28 [1.08, 1.51]	1.23 [1.06, 1.44]
Pure hyperglyceridemia (ICD-9 272.1, n = 1,735)	0.56* [0.18, 1.75]	0.52* [0.07, 3.69]	0.51* [0.13, 2.51]
Mixed hyperlipidemia (ICD-9 272.2, n = 1,921)	2.11 [1.16, 3.83]	2.85 [1.27, 6.39]	1.46* [0.60, 3.53]
Other and unspecified hyperlipidemia (ICD-9 272.4, n = 210,647)	1.00* [0.92, 1.09]	0.83 [0.73, 0.96]	1.07* [0.96, 1.20]
Obesity (multilevel CCS 3.11.2, n = 85,932)	1.14 [1.01, 1.29]	1.24 [1.04, 1.47]	1.03* [0.87, 1.22]
Diabetes with or without complications (single-level CCS 49 and CCS 50, n = 163,474)	0.76 [0.69, 0.84]	0.67 [0.57, 0.79]	0.78 [0.68, 0.89]

The presented trends are based on National Inpatient Sample/Agency for Healthcare Research and Quality (AHRQ) discharges with hip replacement. ORs with 95% CIs including 1 are designated by an asterisk. Metabolic comorbidities were identified using ICD-9 and CCS (AHRQ) codes. Discharges with ICD-9 272.3 code were not available. This table represents numbers of discharge records (not numbers of patients).

CCS, Clinical Classifications Software; CI, confidence interval; ICD-9, International Classification of Diseases revision 9; OR, odds ratio; PO, periprosthetic osteolysis.

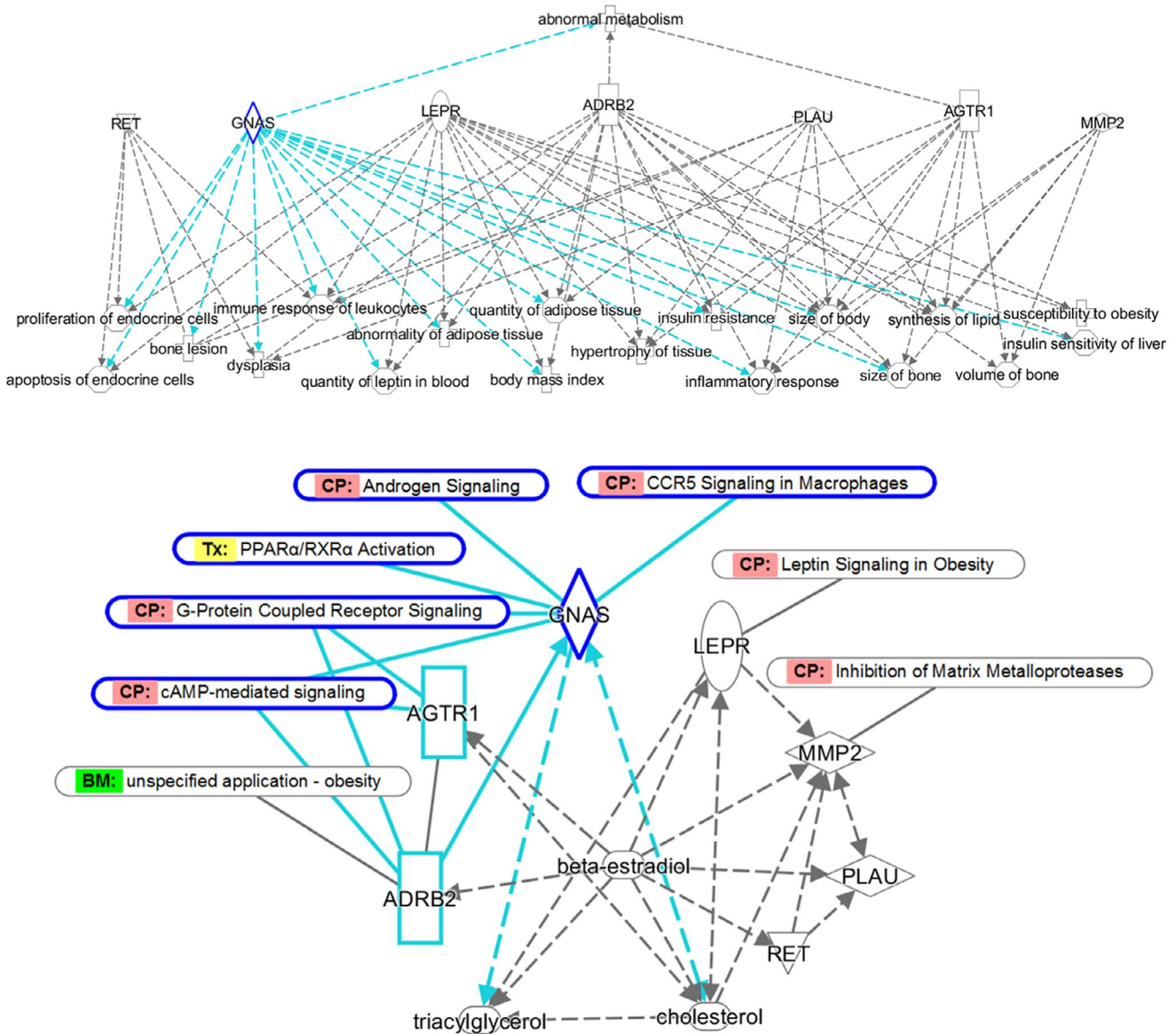


Figure 4 Ingenuity Pathway Analysis (IPA)-based plausibility analysis of candidate single nucleotide polymorphisms (SNPs). (a) Gene functions potentially relevant to bone tissue remodeling and modification by hormonal and metabolic factors. The network was created using IPA knowledge base and IPA core analysis tool and was based on known gene/protein interactions of the genes harboring candidate SNPs. The demonstrated gene/protein-related functions and conditions (with *P* values ranging from 8.15E-09 to 1.03E-05) were selected based on their possible relevance to bone tissue changes and metabolism. Different molecule shapes reflect IPA labels for different gene categories; the arrowed dash lines point at the pathways associated with individual genes/proteins. GNAS-related functions and conditions are highlighted in blue. (b) Gene pathways potentially relevant to bone tissue remodeling and modification by hormonal and metabolic factors. The network was created using IPA knowledge base and IPA core analysis, which was based on known interactions of the genes harboring candidate SNPs. The demonstrated canonical pathways (CP), biomarker (BM) functions, or toxicological (Tx) processes were selected based on their possible relevance to bone tissue changes and metabolism. Different molecule shapes reflect IPA labels for gene/chemical categories. Solid and dash lines reflect direct and indirect interactions, respectively; arrows indicate the direction of interactions, such as activation or inhibition. As an identified upstream regulator, beta-estradiol is capable of regulating expression of almost all network genes (except GNAS) as its downstream targets. A more detailed analysis of GNAS interactions (as highlighted in blue) shows that GNAS is interconnected with both shown lipid species and is particularly capable of affecting cholesterol levels. GNAS and ADRB2 proteins are known to interact directly, and ADRB2 is capable of regulating GNAS protein expression, activation, and localization as well as GNAS binding to GTP. In addition to being part of cAMP/G-protein coupled receptor signaling, GNAS is connected to metabolic (PPARα/RXRα activation) and male sex hormone-related regulation (androgen signaling) as well as to proinflammatory cytokine-mediated pathways (signaling in macrophages). ADRB2, β2-adrenergic receptor 2; LEPR, leptin receptor.

threefold (OR = 2.85; 95% CI = 1.27–6.39), whereas other/unspecified hyperlipidemia (ICD-9: 272.4) decreased PO risk in white women by 17%. Pure hypercholesterolemia (ICD-9: 272.0) moderately increased PO risk in both sex subgroups. Remarkably, diabetes (CCS: 49-50) seemed to reduce the likelihood of PO regardless of sex (by –33% and –22% in women and men, respectively).

Thus, our *ad hoc* epidemiologic RWE on metabolic comorbidities was consistent with the SNP-based evidence on possible sex-dependent metabolic modification of PO risk.

Our further IPA queries associated the genes harboring PO/wear SNP candidates with biofunctions and diseases (Figure 4a) potentially relevant to PO (e.g., bone-related traits) and implant reactivity (e.g., inflammatory/immune responses) as well as endocrine and metabolic effects (e.g., insulin resistance and susceptibility to obesity). Most genes, including GNAS, were linked to biofunctions related to bone morphology.

The genes harboring PO/wear SNP candidates were also connected to signaling pathways (Figure 4b) that can be implicated in implant-related adverse outcomes (e.g., MMP2 in tissue remodeling by matrix metalloproteinases). Metabolic connections were shown by LEPR as the major regulator of leptin signaling and by ADRB2 as a recognized biomarker for obesity. The PO/wear SNP-harboring genes were linked to lipids, such as cholesterol and triglycerides (triacylglycerol). In addition to being regulated by these lipid species, some genes including GNAS were shown to be their upstream regulators. Some PO/wear SNP-harboring genes also showed potential relationships with glucose (not shown).

Reflecting possible influence of sex hormones, beta-estradiol was identified as an upstream regulator of the PO/wear-related gene network ($P = 2.35E-04$, IPA), with almost all SNP-harboring genes as potential downstream targets of beta-estradiol (Figure 4b). The rs7121-harboring GNAS is known to be involved in androgen signaling (Figure S4).

Thus, the integrated genetic and epidemiologic evidence further suggested that the risk of PO in hip replacement may be affected by the sex-dependent metabolic and hormonal factors.

DISCUSSION

As a retrospective study utilizing pre-existing RWD, the current study was subject to inherent limitations, such as the lack of device-related, procedure-related, or patient-related information representing potential confounders. A small sample size and an SNP data set limited to certain disease-related biomarkers were the main limitations of the derived genetic evidence. For example, because almost all PMRP subjects were white, the current SNP findings might not be generalizable to nonwhite subjects. However, many of these limitations are being addressed in our ongoing larger study on discovery of candidate SNPs for adverse outcomes in various joint replacements.

As determined by its focus on developing new methodological approaches to multidisciplinary RWE, our study involved deriving and cross-substantiating two—epidemiologic and genetic—evidentiary streams (Figure 5). First, the AHRQ/NIS-derived epidemiologic RWE revealed an elevated PO risk in white men with hip replacements. Second,

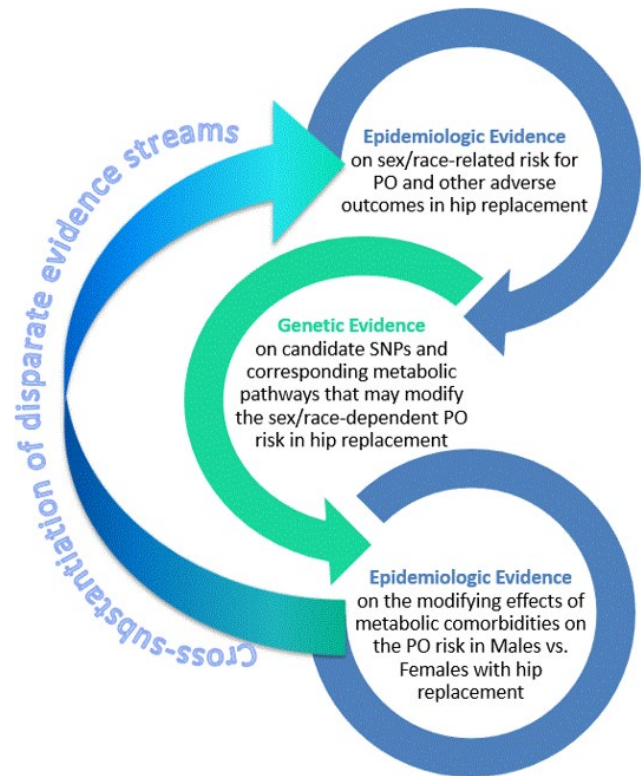


Figure 5 Epidemiologic and genetic evidence streams in *in silico* analysis on the hip replacement-related periprosthetic osteolysis (PO) risk and corresponding candidate single nucleotide polymorphisms (SNPs).

the PMRP-derived genetic RWE and subsequent IPA plausibility analysis identified PO biomarkers whose effects can be modified by metabolic factors. Third, our *ad hoc* AHRQ/NIS queries further corroborated the possibility of sex-related metabolic modification of the PO risk in hip replacement and, thus, substantiated the results of biomarker discovery and subsequent SNP-based analyses.

Sex is recognized as a biological variable that is crucial for mechanistic understanding of disease risk vs. resilience.³⁰ Epidemiologic RWE from our study underscored the need for correct interpretation of the sex-related outcome disparities in hip replacement. Illustrating the methodological importance of sex-based stratification in biomarker discovery, only two of seven candidate SNPs retained their associations with PO or wear in both men and women.

Male sex has been previously associated with the risk for PO,⁶ which was attributed to the higher levels of activity and increased wear. Our study suggested additional reasons for the sex-related differences in PO. The rs7121-harboring GNAS is involved in androgen signaling and is known for its sex-dependent patterns of gene expression. Similar to the study on African American mother-newborn pairs,³¹ in which only maternally transmitted GNAS alleles were associated with birth weight and that association was restricted to male offspring, the male sex-specific association between rs7121 C-allele and PO in our study may indicate the sex-dependent patterns of GNAS splicing and imprinting. Providing further clues

for the GNAS role in the PO risk modification by metabolic factors, alternative murine Gnas gene products were shown to have opposite effects on glucose/lipid metabolism.³² In addition, parental transmission of G-alpha subunit in the knockout murine model was shown to produce opposite—hypermetabolic and hypometabolic—effects that were likely due to deficiency of maternal-specific and paternal-specific Gnas gene products.³³

Obesity has been deemed a relative contraindication for hip replacement and a risk factor for complications and prosthesis failure.³⁴ Although obesity was shown to increase the risk of early revision due to aseptic loosening and/or osteolysis by almost fivefold,³⁵ statins were suggested to reduce femoral osteolysis in hip arthroplasty.³⁶ However, some studies revealed obesity paradox, in which obesity or its correlates provided some protective effect in hip replacement.³⁷ Further, the risk of femoral osteolysis in some normal-weight patients was higher compared with overweight patients.³⁸ Studies on genetically obese mice suggested that obesity may protect against particle-induced bone resorption.³⁹ Although our finding on the reduced PO risk in patients with diabetes with hip replacement seems controversial, similar protective effect was suggested in the nationwide register-based Finnish study,⁴⁰ in which patients with diabetes with hip or knee replacements had slightly decreased hazards ratios for the risk of revision. Further, both obesity and diabetes (*hyperglycemia* in particular) were shown to increase the risk of periprosthetic infection after hip/knee replacement,^{41,42} whereas *hypoglycemia* was shown to increase the risk for revision in knee arthroplasty.⁴³ Thus, further elucidation of metabolic factors in the PO risk should account for possible differences between glucose-related and lipid-related effects.

The role of lipid metabolism in PO has been highlighted by the previously suggested role of SNPs in low-density lipoprotein receptor-related proteins (LRP5 and LRP6).⁹ Although hyperlipidemia may promote osteoclastic potential,⁴⁴ the sex-related differences in energy storage, lipid turnover, and oxidation⁴⁵ (e.g., higher insulin sensitivity in women, or triglyceride synthesis suppression by estrogens) may affect deposition of lipids, such as low-density lipoproteins in subendothelial spaces of the bone,⁴⁶ thereby shaping the sex-dependent host responses leading to PO.

Although the nature of sex-dependent effects on PO remains to be elucidated, it is plausible to suggest that overall hyperlipidemia may activate foreign body responses leading to inflammation and bone tissue remodeling,⁴⁶ whereas the sex-specific metabolic and hormonal differences⁴⁵ may fine-tune distinct PO risks in men and women. The female sex-specific hormonal factors,⁴⁷ such as use of contraceptives and postmenopausal hormone replacement therapy, may further modify the PO risk in women.

In summary, by demonstrating the guiding role of epidemiologic RWE and using unconventional analytics in biomarker discovery, the present study highlighted the potential of *in silico* methodology for optimizing use of medical products and improving treatment outcomes. The sex/race-related trends and candidate SNPs identified in this study can be further corroborated for development of healthcare

measures aimed to reduce the risk of PO as a main orthopedic implant-related complication.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1. Sex/race-related trends in prosthesis-related adverse outcomes.

Figure S2. Female predominance in discharges with hip joint replacement (NIS/AHRQ, 2006–2012).

Figure S3. IPA-based Venn diagram comparing genes harboring candidate SNPs for periprosthetic osteolysis and wear with osteolysis-related genes identified by IPA.

Figure S4. Main pathways derived from the analysis of eQTLs associated with sex-related subclusters of candidate SNPs for periprosthetic osteolysis.

Table S1. List of SNPs used for deriving genetic evidence on putative biomarker candidates for periprosthetic osteolysis.

Table S2. Demographic characteristics of AHRQ/NIS (2010–2014) discharges with hip replacement-related adverse outcomes.

Table S3. eQTLs associated with SNPs from sex-related subclusters with periprosthetic osteolysis.

Table S4. (a) Metabolic complications in the sex/age-stratified white subpopulation with hip arthroplasty and periprosthetic osteolysis (AHRQ/NIS, 2010–2014). (b) Metabolic complications in the sex/age-stratified white subpopulation with hip arthroplasty (AHRQ/NIS, 2010–2014).

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Conflict of Interest. The authors declared no competing interests for this work.

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