Genetic Variation in Inflammasome Signaling and Bone Turnover Pathways and Risk of Osteolysis after Total Hip Arthroplasty

INTRODUCTION:
Loosening of prosthetic components after total hip arthroplasty (THA) due to osteolysis is the leading cause for revision surgery. Regulators of osteoblast and osteoclasts, such as the wnt and RANK/RANKL/OPG pathways, respectively, are thought to play a key role in the development of osteolytic lesions. Recent evidence has also highlighted the role of inflammasome signaling pathways, including the TLRs, NLRP3, purinergic receptors, and MSK1+2 in inflammatory signaling and they may therefore also play an important role in the pathogenesis of osteolysis.

Susceptibility to osteolysis is thought to be modulated by genetic variation such as single nucleotide polymorphism (SNP). Thus variation within genes encoding the regulators of bone formation, including DKK1, KREMEN2, LRP5, LRP6, SFRP1, SOST, and Wnt3A may influence individual risk of osteolysis. Similarly, variation within the RANK/RANKL/OPG regulation of osteoclastogenesis and osteoclast activation may also affect susceptibility to osteolysis. Further, variation within inflammasome signaling genes, including TLR1, TLR2, TLR4, TLR6, TLR9, MD2, NOD1, NOD2, NLRP3, Myd88, TRIF, TRAM, TIRAP, MSK1 and MSK2, P2Y1, P2Y2, P2Y6, and P2X7 may also act as susceptibility loci for osteolysis.

In this 2-stage case-control association study we examined whether variation within these candidate genes are susceptibility loci for osteolysis following THA using a SNP-tagging approach; and also whether these variants act as quantitative trait loci (QTL) for time to prosthesis failure.

METHODS:
Two cohorts, comprising 763 (371 male) Caucasian European patients following THA with a metal on polyethylene bearing couple for primary osteoarthritis, were studied. 318 subjects had either undergone revision surgery for aseptic loosening or had radiographic evidence of osteolysis.

The initial “discovery” cohort comprised 631 patients (275 osteolysis cases) recruited from a North of England population. DNA was extracted from peripheral blood using standard methods. DNA samples were genotyped for candidate genes within the RANK/RANKL, Wnt, P2 purinergic, and PRR pathways, and included 5kb upstream and 2kb downstream of each gene of interest. 318 tagging SNPs were selected using Hapmap Genome Browser and Haploviev software (pair-wise tagging, r²=0.8, MAF>0.05). Following quality control, case-control association analysis and QTL analysis for time to prosthesis failure (in cases only) were performed using an open source whole genome association analysis toolset (PLINK, version 1.07, Harvard University, Boston, MA) and adjusted for age, gender, and time since primary surgery.

A second “replication” cohort of patients, previously recruited from the North West of England, comprised 132 patients (43 osteolysis cases). 18 SNPs that had a P value of <0.05 for either case-control analysis or as a quantitative trait locus for time to failure in stage 1 were carried forward for analysis in the replication cohort and association analysis as for stage 1 was undertaken. A meta-analysis was also undertaken using combined stage 1 and 2 data.

RESULTS:
Stage 1 – Discovery Cohort: Four SNPs within RANK, 2 within TIRAP and 1 each within OPG, KREMEN2 and SFRP1 showed possible association with osteolysis susceptibility (P<0.05). Two SNPs within LRP6, and 1 each within NLRP3, SOST, TRAM, SQSTM1, NOD2, TIRAP AND LRP5 were potential QTLs for time to implant failure (P<0.05).

Stage 2 – Replication Cohort: Case-control and QTL analyses identified no replicated risk loci at P<0.05. However, similar trends were observed for many of the loci compared with stage 1.

Meta-analysis of stage 1+2 Cohorts: Three SNPs within RANK, 2 within TIRAP, and 1 each within SFRP1 and KREMEN2 showed possible association for osteolysis susceptibility (Table 1). Two SNPs within LRP6, and 1 each within NLRP3, TIRAP AND LRP5 were possible QTLs for time to implant failure (Table 2).

DISCUSSION:
Our data suggest that variation within genes encoding inflammasome signaling and bone turnover pathways play a role in susceptibility to osteolysis and time to prosthesis failure after THA. Variation within RANK showed the strongest association with susceptibility to failure and is consistent with the pivotal role of signaling through this receptor as a master regulator for osteoclastogenesis. Variation within NLRP3 was most strongly associated with time to prosthesis failure, and suggests a potential role of variation within inflammasome signaling pathways as a modulator of time to prosthesis failure due to osteolysis. Although some signals in stage 1 showed similar trends in stage 2, small sample size limited confirmation in this dataset. Due to the large number of SNPs tested, and the nature of genetic association tests, these data should be considered preliminary, and further replication in a larger independent population is required before these associations can be confirmed.

SIGNIFICANCE:
The clinical outputs of this study include novel risk loci for osteolysis/ aseptic loosening. Further understanding has the potential for the development of novel biomarkers and treatment molecules.

ACKNOWLEDGEMENTS:
This study was funded by a grant from the Sheffield NIHR Musculoskeletal Biomedical Research Unit.