CONCISE REPORT

Genetic susceptibility to total hip arthroplasty failure: a preliminary study on the influence of matrix metalloproteinase 1, interleukin 6 polymorphisms and vitamin D receptor

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Background: Matrix metalloproteinase (MMP)1, interleukin(IL)6 and vitamin D receptor (VDR) have been implicated in the biological cascade of events initiated by particulate wear debris and bacterial infection, resulting in periprosthetic bone loss around loosened total hip replacements (THRs). The individual responses to such stimuli may be dictated by genetic variation.
Objective: To study the effect of single-nucleotide polymorphisms (SNPs) within these candidate genes.
Methods: A case–control study of the MMP1, IL6 and VDR genes was performed for possible association with deep sepsis or aseptic loosening. All cases included in the study were Caucasian patients with osteoarthritis who had received a cemented Charnley total hip arthroplasty (THA) and polyethylene acetabular cup. Cases consisted of 91 patients with early aseptic loosening and 71 patients with microbiological evidence of deep infection on surgery. Controls consisted of 150 patients with THAs that were clinically asymptomatic for over 10 years and showed no radiographic features of aseptic loosening. DNA samples from all individuals were genotyped using Taqman allelic discrimination.
Results: The C allele ($p = 0.001$; OR = 3.27; 95% CI 2.21 to 4.83) and C/C genotype ($p = 0.001$) for the MMP1 SNP were highly associated with aseptic failure when compared with controls. No statistically significant relationships were found between aseptic loosening and the MMP2, MMP4, IL6 −174 or VDRL SNPs. The T allele ($p = 0.007$; OR = 1.76; 95% CI 1.16 to 2.66) and T/T genotype ($p = 0.028$) for VDR-T were statistically associated with osteolysis owing to deep infection as compared with controls. No statistically significant relationship was found between septic failure and any of the other SNPs examined in this study.
Conclusions: Aseptic loosening and possibly deep infection of THR may be due to the genetic influence of candidate susceptibility genes. SNP markers may serve as predictors of implant survival and aid in pharmacogenomic prevention of THR failure.

Total hip replacement (THR) is a cost-effective and highly effective surgical intervention.[1] The long-term success of this procedure is limited by wear of the articulating interfaces of the implants used and the subsequent adverse biological response to the wear debris generated.[2] The principal mechanism of failure is loosening of the implants and periprosthetic osteolysis, otherwise termed aseptic loosening.[3–7] Deep infection of THR is the commonest cause of early failure.[8] The mechanism by which bacteria establish periprosthetic infection and cause bone damage is currently poorly understood, although a recent study has suggested that stimulation of osteoclastic bone resorption is central to the loss of bone seen in deep infection.[9]

Susceptibility to osteolysis is likely to be due to a combination of environmental and genetic factors, as progression of loosening and osteolysis varies between individuals with apparently identical total joint replacements. Environmental factors have been extensively studied and can explain the onset of osteolysis in certain situations.[10–12] What strengthens the belief that biological factors play a great role in determining survival or failure of an implant is that prostheses with very different design concepts, surface finish and biomechanical properties within the cement mantle[13–15] have similar survival rates. Initial diagnosis, age, sex and weight have been shown to have only a slight effect upon implant survival.[16–18]

Tissue retrieval studies indicate that the membranous tissue that surrounds loose THR prostheses plays a central role in the development of osteolysis.[19] The histopathological findings are those of a fibrous stroma, abundant macrophages, foreign body giant cells and wear debris.[20 21] These cells produce multiple proinflammatory factors that are strongly implicated in periprosthetic osteolysis such as tumour necrosis factor (TNF)α, interleukin 6 (IL6) and matrix metalloproteinases (MMPs).[20 22 23] Immunohistochemical analysis has shown MMP1 to be consistently present.[24] The presence and activity of MMP1 in such tissue has been confirmed by expression and enzymatic activity studies, respectively.[25 26] IL6 has been shown to be strongly expressed in the interface membrane around loosened THRs.[27]

MMP1 is a protease that breaks down the interstitial collagens I, II and III. Recently it has become evident that it has a number of other substrates such as cytokines (eg, pro TNFα) and growth factor precursors and binding proteins (eg, perlecan).[28] MMP1 gene variation has also been shown to be associated with increased transcription factor production, metastasis, invasiveness of cancers and premature rupture of fetal membranes.[29]

IL6 is a pleiotropic cytokine involved in the regulation of bone metabolism, the immune response and the acute-phase response.[30] The transcriptional activity of the gene coding for IL6 and the plasma levels of IL6 are associated with a single G/C base exchange SNP at −174 position of the 5′ flanking region of the IL6 gene.[31] This −174 SNP has been shown to be associated with numerous diseases.[32–37]

Regional periprosthetic bone loss occurs in well-fixed THAs both in the early period after implantation[38 39] and in the later period (>10 years after implantation).[40] The role of region-specific decreases in bone mineral density (BMD) has been related to the failure of cemented femoral components.[40]

Abbreviations: BMD, bone mineral density; IL, interleukin; MMP, matrix metalloproteinase; SNP, single-nucleotide polymorphism; THA, total hip arthroplasty; THR, total hip replacement; TNF, tumour necrosis factor; VDR, vitamin D receptor

Analysis of the Norwegian Arthroplasty Register data has suggested a higher incidence of aseptic loosening in THA performed for femoral neck fracture[16] and in patients taking corticosteroids.[41] These relationships suggest that the risk of aseptic loosening may be linked to regional and localised decrease in BMD as both are strong risk factors for osteoporosis.

A relationship between BMD and variation in the VDR gene has been described.[42 43] Subsequent studies have shown an association between VDR alleles[44] and decrease in postmenopausal BMD and increase in BMD after vitamin D treatment for osteoporosis.[45] However, the clinical effect of these variants remains unclear as conflicting reports have been published with no clear consensus becoming apparent. Most studies have concentrated on investigating the BsmI, TaqI and ApaI restriction site polymorphisms which are all found in non-coding regions of the VDR gene. These have no effect on the protein product of the gene, but may be in linkage disequilibrium with SNPs that have an effect on BMD.[46] Within exon 2 of the VDR gene, two polymorphisms exist (VDR-L, which is a T-C transition; and VDR-T, which is a G–T transition), which are present in codon 1. These result in a shorter product of translation and may cause a change in the biological activity of the protein.[47]

In this study, we have tested the hypothesis that there is an association between MMP1 SNPs, two proposed functional variations in the VDR gene and the IL6 G/C functional polymorphism, and the occurrence of osteolysis or deep infection around a joint arthroplasty.

PATIENTS AND METHODS

Patients

All patients were recruited from outpatient clinics at our hospital. Strict inclusion and exclusion criteria were used for all groups to make them directly comparable. Only Caucasian patients from the north west of England of either sex with a primary diagnosis of osteoarthritis were included. All had a cemented Charnley monoblock femoral stem and either a cemented Charnley or an Ogee cup.

The aseptic group consisted of subjects who had early loosening of prosthetic implants. Early aseptic loosening was defined as that occurring within 6 years of implantation in line with the yearly incidence of loosening in relation to the evolution of the Charnley low friction arthroplasty.[48] None had clinical, biochemical or operative findings suggestive of infection. Femoral stem aseptic loosening was defined by findings at revision surgery, the definite radiographic loosening criteria of Harris[49] or progressive endosteal cavitation across zones as described by Gruen.[50] Demarcation of acetabular components was recorded according to the zones described by DeLee and Charnley,[51] and loosening confirmed either at the time of revision surgery or by using the criteria of Hodgkinson *et al*.[52] All the parameters above are well-accepted and validated indicators of aseptic failure of total hip components and have been used widely in the orthopaedic literature. In total, 69 of the aseptic cases recruited had this diagnosis confirmed at the time of revision surgery as well as radiographically.

Well-fixed control THAs were defined as those that had remained clinically asymptomatic for over 10 years and showed no radiographic features of aseptic loosening as described above, or ''atrisk'' signs as described by Pachecho *et al*.[48]

The septic (deep infection) group consisted of subjects who had proved deep bacterial infection at the time of revision surgery. All cases had at least three positive cultures of the same bacterium from different samples taken at the time of revision surgery.

In total, 312 patients were recruited to the trial (150 controls, 91 aseptic and 71 septic). The mean age of all patients was 68.6 years (70.9 in the control group, 66.2 in the aseptic group and 68.7 in the septic group). The differences in age and sex between the groups were not statistically significant.

Average length of survival for controls was 14.6 years. Average time to aseptic loosening was 5.1 years. Average time to first diagnosis of infection (either clinical or radiological) was 0.83 years. Infecting organism was a coagulase-negative Staphylococcus in 69%, *Staphylococcus aureus* in 14%, Streptococcus in 6%, *Escherichia coli* in 6% and other bacteria in 5%.

DNA extraction

About 5 ml of peripheral venous blood was collected in EDTA from each subject. DNA was extracted from pelleted white blood cells using phenol and ethanol. Concentrations of DNA were measured using a picogreen assay technique and diluted to 20 ng/μl.

Genotyping

All SNPs were typed using the *Taq*Man allelic discrimination assay[53] developed for use on the 7700 instrument (PE Applied Biosystems). Polymerase chain reactions (PCRs) were performed in 5 μl reaction volumes in 96-well plates and contained 10 ng genomic DNA per reaction. The standard protocol provided with the kit was followed. Thermocycler conditions were an initial 10 min denaturation at 95℃, followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min. Reporter fluorescence emission was detected by an AB7700 fluorescent plate reader using Sequence Detection Software (Applied Biosystems, Forster City, California, USA) for allele calling.

Four common polymorphisms of MMP1 (termed MMP1-1, MMP1-2, MMP1-3 and MMP1-4) were typed. For the first three SNPs, assay-by design *Taq*man assay reagent kits (PDAR) containing one pair of PCR primers and one pair of fluorescently labelled Taqman probes were used (Applied Biosystems). For the MMP1-4 SNP, an assay-by demand (Applied Biosystems reference number 632722) was used, details of which can be found at the company website (http://celera.com MMP1–4 (RS470747) is a C/T SNP in intron 8.

MMP1-1 (RS5854) is a T/C SNP in the 3′ untranslated region. Probe sequences used were VIC-TAC TCT GGA AAA GAT C-MGBNFQ and 6FAM-CAC ATC TTG CTC TTG T-MGBNFQ. Forward and reverse primers were GAT TCA TAT AGG CCA GAG TTG CAA A and GAA GCT GCT CTC TGG GAT CAA C. MMP1–2 (RS554499) is a T/G SNP in exon 2. Probe sequences used were VIC-CAT CTG GCT CTT GTG-MGBNFQ and 6FAM-CAC ATC TTG CTC TTG T-MGBNFQ. Forward and reverse primers were GGC AGC ATT TAC CTG GAC TAA GTC and AGC TTC CCA GCG ACT CTA GAA A. MMP1-3 (RS2397776) is a C/T SNP in exon 6. Probe sequences used were VIC-ACC AGT GAC AGG AGG-MGBNFQ and 6FAM-ACC AGT GAT AGG AGG G-MGBNFQ. Forward and reverse primers were GGA ACA TCC AAC AAA AGA AAC ACA and CAC TCC TGG ACC TTG GAG GAA.

The IL6 –174 SNP was typed using an assay-by design *Taq*man assay reagents kit. Probe sequences used were VIC-CCT TTA GCA TCG CAA GAC-MGBNFQ and 6FAM-CTT TAG CAT GGC AAG AC-MGBNFQ. Forward and reverse primers were GAC GAC CTA AGC TGC ACT TTT C and GGG CTG ATT GGA AAC CTT ATT AAG ATT G.

The VDR-L, and VDR-T SNPs were typed using an assay-by design *Taq*man assay reagents kit. For VDR-L, probe sequences used were VIC-TCA TGT TGC GCT CCA A-MGBNFQ and 6FAM-CAT CAT GTT GCT CTC CAA-MGBNFQ. Forward and reverse primers were CTG CTG AAG TCA AGT GCC ATT G and GCC ACA GGT CCA GGA CAT G. For VDR-T, probe sequences used were VIC-ATT GCC TCC ATC CCT GT-MGBNFQ and 6FAM-TGC

CTC CGT CCC TGT-MGBNFQ. Forward and reverse primers were TGG CCT GCT TGC TGT TCT T and GGG TCA GGC AGG GAA GTG.

Statistical analysis

Power calculations performed for each SNP, based on the known frequency of each allele in known sample Caucasian populations showed that the number of samples collected in this study would have 80% power, with a 5% type 1 (α) rate to detect statistically significant differences between cases and controls.

For each SNP the STATA V.7.0 statistical analysis program was used to calculate the distribution of allele frequencies between subjects and controls, and χ^2 test for comparisons . Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated.

RESULTS

The genotype distribution was in Hardy–Weinberg equilibrium for all SNPs tested in both cases and controls. Allele and genotype frequencies are given in table 1 for MMP1 SNPs and in table 2 for IL6 and VDR SNPs. The MMP1-2 and VDR-L SNPs proved to be monomorphic in all groups and as such are not represented in the tables.

Differences in the number of subjects in the two tables are because of the failure of small numbers of reactions during the genotyping processes for individual SNPs.

The frequencies of the C allele ($p = 0.001$; OR = 3.27; 95% CI 2.21 to 4.83) and C/C genotype ($p = 0.001$) for the MMP1 SNP were highly associated with aseptic failure as compared with controls. No statistically significant relationship was found between aseptic loosening and the MMP2, MMP4, IL6 –174 or VDR-L SNPs.

The T allele ($p = 0.007$; OR = 1.76; 95% CI 1.16 to 2.66) and T/T ($p = 0.028$) genotype for VDR-T were significantly associated with osteolysis owing to deep infection as compared with controls. No statistically significant relationship was found between septic failure and any of the other SNPs examined in this study.

Table 1 Matrix metalloproteinase 1 allele and genotype frequencies in controls, aseptic and septic total hip replacement failures (numbers in brackets)

SNP	Control	Aseptic	Septic
MMP1–1			
Number of subjects	148	87	62
Allele frequency			
T	0.66 (194)	0.37 (64)	0.68 (84)
C	0.34 (102)	0.63 (110)	0.32 (40)
Genotype frequency			
T/T	0.41 (61)	0.17 (15)	0.47 (29)
T/C	0.49 (72)	0.39 (34)	0.42 (26)
C/C	0.1 (15)	0.44 (38)	0.11 (7)
MMP1–3			
Number of subjects	148	88	62
Allele frequency			
C	0.79 (234)	0.76 (134)	0.83 (102)
T	0.31 (62)	0.24 (42)	0.17 (22)
Genotype frequency			
C/C	0.62 (92)	0.55 (48)	0.68 (42)
C/T	0.34 (50)	0.43 (38)	0.29 (18)
T/T	0.04 (6)	0.02 (2)	0.03 (2)
MMP1–4			
Number of subjects	147	89	62
Allele frequency			
C	0.65 (190)	0.67 (119)	0.69 (86)
T	0.35 (104)	0.33 (59)	0.31 (38)
Genotype frequency			
C/C	0.40 (59)	0.48 (43)	0.45 (28)
C/T	0.49 (72)	0.37 (33)	0.48 (30)
T/T	0.11 (16)	0.15 (13)	0.07 (4)

MMP, matrix metalloproteinase.

DISCUSSION

Excellent outcomes have been extensively reported following THR, but the long-term failure of these implants due to loosening and associated bone loss remains a problem.[54] A popular hypothesis is that wear particles induce a chronic inflammatory reaction by directly stimulating monocytes and macrophages to release numerous proinflammatory cytokines, and subsequently increase osteoclast-mediated bone resorption.[19 22]

The effect of MMP1 may be produced by the direct degradation of the extracellular organic matrix of bone. This is the case with regard to connective tissue destruction and remodelling associated with metastasis, cartilage destruction and the development of aneurysms.[28] Recent data provide convincing evidence that its substrates include molecules involved in cell migration, invasion and morphogenic responses to extracellular stimuli.[55] Deep infection continues to be the most feared complication of THR surgery.[56] In preventing sepsis, surgical technique, theatre design and antibiotic usage are important.[57] However, a significant number of patients develop joint infections without obvious predisposing causes. The mechanism by which bacteria establish periprosthetic infection and cause bone damage is poorly understood, with little knowledge available on specific aspects of patients' cytokine and cellular responses to periprosthetic infection. Local production and activation of MMP1 and its subsequent action on substrates involved in inflammation and neovacularisation such as proTNFα and CXCL 12 may suggest its role in the development of deep sepsis around THR.

We have shown an association between the MMP-1 SNP and THR aseptic failure. This SNP exists within a promoter region of the gene and as such may have a direct effect on the amount of gene expression.[58] However, the mechanisms of MMP gene regulation are still not fully delineated, and it is likely that many more functionally important elements in the promoter region are yet to be identified.[29 59 60] Regarding MMPs solely as agents of extracellular matrix destruction may be too simplistic.[28] Proteolysis of extracellular matrix components can affect cell–cell communication and cell apoptosis. In addition, a number of non-matrix substrates that potently affect cellular function have been identified. Degradation products of collagen and matrix components are chemotactic for monocytes and may lead to the accumulation of monocytes and macrophages in the periprosthetic connective tissues. Many MMPs play protective and anti-inflammatory roles. Therefore, the association that exists with a particular polymorphic form of MMP1 does not show that that particular form is associated with increased MMP1 activity; in fact, the opposite may be true.

IL6 expression has been shown by in vivo, in vitro and tissue explant studies to be upregulated in the periprosthetic interface loosening membrane.[19] The level of IL6 in the serum of patients with aseptic loosening has been shown to be altered by variations in type of implant used and cementation technique.[61] IL6 stimulates osteoclast activity and bone resorption by an indirect mechanism owing to osteoblastic production of downstream effectors such as RANKL.[30]

Table 2 Vitamin D receptor and interleukin 6 allele and genotype frequencies in controls, aseptic and septic total hip replacement failures (numbers in brackets)

	Control	Aseptic	Septic
VDR-T SNP			
Number of subjects	148	88	63
Allele frequency			
G	0.60 (178)	0.64 (112)	0.47 (59)
T	0.40 (118)	0.36 (64)	0.53 (67)
Genotype frequency			
G/G	0.37 (55)	0.40 (35)	0.25 (16)
G/T	0.46 (68)	0.48 (42)	0.43 (27)
T/T	0.17 (25)	0.12 (11)	0.32 (20)
SNP IL6 –174			
Number of subjects	149	87	63
Allele frequency			
T	0.60 (178)	0.58 (101)	0.58 (73)
C	0.40 (120)	0.42 (73)	0.42 (53)
Genotype frequency			
T/T	0.39 (57)	0.35 (31)	0.29 (18)
T/C	0.43 (64)	0.44 (39)	0.59 (37)
C/C	0.18 (28)	0.21 (17)	0.12 (8)

IL, interleukin; SNP, single-nucleotide polymorphism; VDR, vitamin D receptor.

Both septic and aseptic failure lead to periprosthetic bone loss, and the –174 SNP has been shown to be associated with decreased BMD.[36 37] The functional significance of this promoter polymorphism remains to be established. Transcription of the gene may not be by simple additive effects, but rather by complex regulatory effects determined by haplotype associations. As such, we intend to examine more SNPs of potential functional importance within the IL6 gene.

In the current literature, there are discrepancies between studies regarding the relationships between VDR genotypes and bone metabolism, and the true influence of VDR is yet to be determined.[62] Our study has shown no association between variation within exon 2 of the VDR gene and aseptic loosening of THR, but has shown an association with bone loss secondary to deep infection. This may be due to differing mechanisms of bone loss and repair between these two modes of failure.[5] The vitamin D endocrine system plays an important role in bone cell physiology and may have an immunosuppressive effect[63] as well as directly modulating the recently discovered RANK/RANKL/OPG triad which acts as the primary control over osteoclast differentiation and activation.[30] As such, an alteration in the protein product of VDR may adversely affect the bone response to bacterial challenge in the presence of prosthetic implants and particulate debris. In vivo studies seem to confirm that the responsiveness to 1,25-dihydroxyvitamin D3 on bone metabolism varies according to the variation within the VDR gene.[64]

Association studies such as the one described in this report can be very useful in elucidating the relationship between complex disease phenotype and a specific genotype. However, they have a number of caveats such as small sample size, population stratification and linkage disequilibrium. We have managed to collect a large number of study and control cases, with approximately twice as many controls as study subjects in the aseptic group. Slightly fewer septic cases were recruited, which probably represents the lower prevalence of this complication. We recognise the statistical advantages of having as large study groups as possible and are continuing to increase our sample size. One difficulty with this is that we have set such stringent recruitment criteria in an attempt to avoid population stratification and clinical heterogeneity.

The findings of this study are novel and will require verification by further studies. In particular, we aim to perform in vitro cell studies to determine the functional effects of allelic variants on cellular response to wear particles and to establish whether these effects are manifested at the gene expression level or proteomic level. In addition, we have begun a prospective survivorship analysis of patients undergoing THR to quantify the association between risk factors as described in this study and those described in a previous study and time of failure.

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