Genetic Susceptibility to Total Hip Arthroplasty Failure—Positive Association With Mannose-Binding Lectin

Mohammad H.A. Malik, MA, FRCS (T&O),*† Ardeshir Bayat, PhD,† Francine Jury, MSc,† Peter R. Kay, BSc, FRCS,* and William E.R. Ollier, PhD, FRCPath†

Abstract: Mannose-binding lectin (MBL) may be involved in the biologic cascade of events initiated by wear debris and bacterial infection around loosened total hip arthroplasties (THAs). Individual responses to such stimuli may be dictated by genetic variation caused by single nucleotide polymorphisms (SNPs). We performed a case-control study on 4 MBL SNPs using case patients (n = 91) with aseptic loosening or deep infection (n = 71). Control subjects (n = 150) had clinically and radiologically well-fixed THAs for more than 10 years. Frequency of the C allele (P = .001) and that of the genotype C/C (P = .004) for the −550 SNP were associated with aseptic failure. The codon 54 SNP G allele (P = .012) and G/G genotype (P = .027) frequencies were associated with aseptic failure as well. In the septic group, the frequency of the C allele (P = .01) and that of the genotype C/C (P = .05) for the −550 SNP were significant. Failure of THAs may be under genetic influence to candidate susceptibility genes such as MBL. **Key words:** total hip arthroplasty, gene, failure, infection, association.

Total hip arthroplasty (THA) has restored function and mobility to patients who previously had very little in the way of effective treatment options before its invention [1]. Unfortunately, failure in a certain proportion can be expected over time. A figure of less than 10% at 10 years is quoted as the standard for joint arthroplasties with well-documented follow-up [2]. The principal causes are loosening of the implants and periprosthetic osteolysis [3], otherwise termed *aseptic loosening*. Osteolysis is believed to be a biologic response to a variety of particles that may originate at several locations around a joint arthroplasty. These include the articulating surfaces, fixation surfaces, modular component interfaces, and devices used for adjuvant fixation [4,5].

The effects of fixation method, surgical technique, and implant design have been clearly demonstrated in the Swedish National Hip Register of 92 675 total joint arthroplasties [6]. The introduction of third-generation cementation techniques and improved surgical technique has led to a 10% to 17% reduction in the revision rate. Strikingly, aseptic loosening accounted for 75.7% of all revisions and deep infection did for 7.2%, whereas technical error only accounted for 3.5%. What strengthens the belief that biologic factors play a great role in

*From the *Center for Hip Surgery, Wrightington Hospital, Wigan, United Kingdom; and †Center for Integrated Genomic Research, The University of Manchester, Manchester, United Kingdom.*
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Reprint requests: Mohammad H.A. Malik, MA, FRCS (T&O), Center for Integrated Genomic Research, The University of Manchester, Stopford Bldg, Oxford Road, Manchester M13 9TP, UK.

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determining the survival or failure of an implant is the observation that prostheses with very different design concepts, surface finish, and biomechanical properties within the cement mantle have almost identical survival rates [7,8].

Deep infection is the commonest cause of early failure [9]. Prosthetic infection often arises as a result of gram-positive skin flora of low virulence (eg, *Staphylococcus aureus*, coagulase-negative staphylococci), although other organisms may be involved [10]. In preventing sepsis, issues of surgical technique, theater design, and antibiotic use are very important. There are, however, a significant number of patients who develop joint infections without obvious predisposing causes. The mechanism by which bacteria establish periprosthetic infection and cause bone damage is currently poorly understood, with little knowledge available on specific aspects of patients' cytokine and cellular responses to periprosthetic infection.

Mannose-binding lectin (MBL) is a liver-derived serum protein and is increased approximately 4-fold during the acute-phase response to infection. It belongs to the collectin group of lectins that have a collagen-like structural domain. It possesses the ability to opsonize bacteria, activate both the classic or alternative complement pathways, and activate macrophages via the C1q receptor [11]. It appears that MBL plays an important role in the innate immune system and possibly in chronic inflammation [12]. Mannose-binding lectin deficiency and low serum levels are strongly associated with the presence of 4 single nucleotide polymorphisms (SNPs) within the MBL gene on chromosome 10 [13]. Formation of a triple helix in the collagenous tail of the protein product is impaired by mutation in codons 52 and 54, and this disrupts polymerization and causes functional deficiency of MBL. Interindividual variation in serum MBL levels has also been linked to promoter SNPs at positions −221 and −550 [14]. Clinical studies have demonstrated these SNPs to be associated with susceptibility to infection in systemic lupus erythematosus [14], bacterial sepsis [15], immunodeficiencies [16], inflammatory arthritis [17], and inflammatory bowel disease [18].

Because virtually all deep sepses encountered in THA surgery are bacterial, it is probable that MBL plays a central role in the host innate response to this complication [19]. Recently, it has been shown that certain implanted polymers may provide an adjuvant-like activity to native macromolecules, which subsequently become immunogenic. This suggests that ultra–high-molecular-weight polyethylene–bound proteins may represent antigens to preexisting antibodies in patients who have had a THA and provide a mechanism for their removal by phagocytosis once they have been bound by MBL [20,21]. One study suggested that immunoglobulin complexed with polyethylene may fix complement, which the authors postulated may in turn attract inflammatory cells and so initiate the development of the loosening membrane or interface tissue found in aseptic failure and associated osteolysis of THAs [22]. This would suggest a mechanism for the involvement of MBL in the aseptic loosening process. In this study, we investigated the association between MBL deficiency as associated with SNPs at codons 52 and 54 as well as at promoter positions −550 and −221 and the development of aseptic loosening or deep infection around THAs.

Patients and Methods

Patients

All patients were recruited from outpatient clinics at the Wrightington Hospital (Wigan, UK) between August 2002 and August 2004. All subjects were provided with a detailed information sheet regarding the proposed study and signed a study-specific consent form, both of which had been included as part of the research protocol approved by the Wrightington, Wigan, and Leigh Research Ethics Committee.

Strict inclusion and exclusion criteria were set for the study groups (patients with aseptically loosened or deeply infected THAs) and the control group (subjects with well-fixed implants) to make them directly comparable. Only white patients from Northwest England of either sex with a primary diagnosis of osteoarthritis were included. All had a primary cemented Charnley monoblock femoral stem and either a cemented Charnley or Ogee flanged polyethylene acetabular cup. All the cups were made from an identical type of high-molecular-weight polyethylene provided by a single manufacturer. All patients with hip joint degeneration as a result of secondary causes and inflammatory or septic arthritis were excluded.

The aseptic study group consisted of patients who had early loosening of prosthetic implants. Early aseptic loosening for both components 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 [23]. None had clinical, hematologic, 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 et al [24], or progressive endosteal cavitation across zones as described by Gruen et al [25]. Demarcation of acetabular components was recorded as per the zones described by DeLee and Charnley [26], and loosening was confirmed either at the time of revision surgery or by using the criteria of Mullroy and Harris [8]. Well-fixed control THAs were defined as those that had remained clinically asymptomatic for more than 10 years and demonstrated none of the described radiographic features of aseptic loosening as previously described or at-risk signs as described by Pacheco et al [23].

The septic (deep infection) group consisted of patients who had developed proven deep bacterial infection of a THA (as determined by clinical and microbiologic findings) at the time of revision surgery.

In total, 312 patients were recruited to the trial (150 control subjects and 91 aseptic and 71 septic case patients). The differences between numbers recruited and those genotyped and differences in numbers between different SNPs typed in each study or control group were caused by technical problems in genotyping some of the samples.

The mean age of all patients was 68.6 years (70.9 years in the control group, 66.2 years in the aseptic group, and 68.7 years in the septic group). The differences in age and sex between the groups were not statistically significant. The average length of THA survival for control subjects was 14.6 years. The average time to aseptic loosening was 5.1 years. The average time to first diagnosis of infection (either clinical or radiologic) was 0.83 years. The infecting organism was a coagulase-negative staphylococcus in 69%, *S. aureus* in 14%, streptococcus in 6%, *Escherichia coli* in 6%, and other bacteria in 5%.

DNA Extraction

Five milliliters of peripheral venous blood was collected from each subject into EDTA bottles by a standard venesection technique. All blood samples were stored in a −20°C freezer before DNA extraction. DNA was extracted from pelleted white blood cells using chloroform and ethanol. Concentrations of DNA were measured using a PicoGreen assay technique and diluted to 20 ng/μL with sterile water.

Genotyping

A genotyping assay based on the SNaPshot (PE Applied Biosystems, Warrington, UK) ddNTP primer extension method for these 4 SNPs has already been described in detail [27]. Polymerase chain reaction (PCR) conditions, PCR product purification, and extension steps were followed in all respects in this study. Briefly, the assay involved an amplification step to derive a PCR product containing the polymorphic residue, followed by an allele-specific extension step. Primer sequences and fragment sizes were as follows: for the −550 and −221 SNPs, a 447–base pair (bp) fragment was amplified using the forward primer CCA GGG CCA ACG TAG TAA GA and the reverse primer GAG GGG TTC ATC TGT GCC TA; for codons 52 and 54, a 201-bp fragment was amplified using the forward primer TGG CAG CGT CTT ACT CAG AA and the reverse primer CAG GCA GTT TCC TCT GGA AG. Because of the close proximity of the respective promoter and codon SNPs, only one PCR was required for each pair. The product of both PCRs was then multiplexed during the extension step. The G/C −550 SNP was isolated using forward and reverse probe sequences of GCT TAC CCA GGC AAG CCT GT and TGC TTC CCC TTG GTG TTT TA; the G/C −221 SNP, using forward and reverse probe sequences of ACG GTC CCA TTT GTT CTC ACT GCC AC and TGC TGG AAG ACT ATA AAC ATG CTT TC; the C/T codon 52, using forward and reverse probe sequences of CAG GCA TCA ACG GCT TCC CAG GCA AAG ATG GG and TGG TTC CCC CTT TTC TCC CTT GGT GCC ATC AC; and the G/A codon 54, using forward and reverse probe sequences of AGG CAT CAA CGG CTT CCC AGG CAA AGA TGG GCG TGA TG and CCC AAC ACG TAC CTG GTT CCC CCT TTT CTC CCT TGG TG. Genotyping was performed on an ABI Prism 3100 machine (Applied Biosystems, Foster City, Calif) using GeneScan Analysis software (Applied Biosystems) and viewed using Genotyper 3.6 software (Applied Biosystems).

Statistical Analysis

For each SNP, the STATA 7.0 statistical analysis program (STATA Corporation, College Station, Tex) was used to calculate the distribution of allele frequencies between case patients and control subjects, compared using a χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated.

Results

Genotype distribution was in Hardy-Weinberg equilibrium for all 4 SNPs in both case patients and control subjects. Allele and genotype frequencies for controls and aseptic and septic failures are shown in Table 1.

Table 1. Single Nucleotide Polymorphism Allele and Genotype Frequencies in Controls and in Aseptic and Septic THA Failures (Numbers in Brackets)

SNP	Control	Aseptic	Septic
221			
Subjects (n)	145	91	62
Allele frequency			
G	0.32 (94)	0.34 (62)	0.41 (51)
C	0.68 (196)	0.66 (120)	0.59 (73)
Genotype frequency			
G/G	0.08 (12)	0.11 (10)	0.15 (90)
G/C	0.48 (70)	0.46 (42)	0.53 (33)
C/C	0.44 (63)	0.43 (39)	0.32 (20)
550			
Subjects (n)	148	91	61
Allele frequency			
G	0.85 (251)	0.71 (130)	0.74 (90)
C	0.15 (45)	0.29 (52)*	0.26 (32)†
Genotype frequency			
G/G	0.73 (108)	0.54 (49)	0.54 (33)
G/C	0.24 (35)	0.35 (32)	0.39 (24)
C/C	0.03 (5)	0.11 (10)‡	0.07 (4)§
52			
Subjects (n)	148	91	62
Allele frequency			
C	0.91 (268)	0.93 (170)	0.94 (116)
T	0.09 (20)	0.07 (12)	0.06 (8)
Genotype frequency			
C/C	0.84 (124)	0.87 (79)	0.89 (55)
C/T	0.16 (24)	0.13 (12)	0.10 (6)
T/T	0 (0)	0 (0)	0.01 (1)
54			
Subjects (n)	144	91	62
Allele frequency			
G	0.84 (241)	0.92 (167)†	0.90 (111)
A	0.16 (47)	0.08 (15)	0.10 (13)
Genotype frequency			
G/G	0.68 (98)	0.84 (76)‖	0.82 (51)§
G/A	0.31 (45)	0.16 (15)	0.15 (9)
A/A	0.01 (1)	0 (0)	0.03 (20)

*P = .001.
†P = .01.
‡P = .004.
§P = .05.
‖P = .03.

The frequency of the C allele (P = .001; OR = 2.23; 95% CI = 1.42-3.50) and that of the genotype C/C (P = .004) for the −550 SNP were highly associated with aseptic failure as compared with controls. In addition, the codon 54 SNP G allele (P = .012; OR = 2.17; 95% CI = 1.18-3.98) and G/G genotype (P = .027) frequencies were associated with aseptic failure. No statistically significant relationship was found between aseptic loosening and the promoter −221 or codon 52 SNPs.

With regard to the septic group, as compared with the control group, the frequency of the C allele (P = .01; OR = 1.90; 95% CI = 1.14-3.16) and that of the genotype C/C (P = .05) for the −550 SNP were statistically significant. Only the G/G genotype frequency (P = .05; OR = 1.47; 95% CI = 0.79-2.72) of the codon 54 SNP was statistically significant. The allele frequency was not associated with septic failure of THA. No statistically significant relationship was found between septic failure and the promoter −221 or codon 52 SNPs.

Discussion

The goal in gaining a better understanding of the mechanism of osteolysis is to reduce wear-related complications after total joint arthroplasty. This can be approached by efforts to reduce particle generation and to inhibit the biologic response to particulate debris. Susceptibility to osteolysis is probably caused by a combination of environmental and genetic factors as progression of loosening and osteolysis varies between individuals with apparently identical total joint arthroplasties. The former has been extensively studied and can explain the onset of osteolysis in certain situations [28].

Recent research have been directed at understanding the biologic cascade of events that is initiated by particulate debris and results in periprosthetic bone loss. A large number of studies have documented the release of a variety of mediators of bone resorption from periprosthetic tissues in tissue culture [21]. The histopathologic features of the periprosthetic membrane have been studied extensively. Common findings in cases of aseptic loosening or osteolysis include a fibrous stroma, abundant macrophages, foreign-body giant cells, and wear debris. In cases of deep infection, there is a proliferation of polymorphonuclear and B cells. Analysis of these interface membranes has demonstrated the presence of many biochemical mediators of inflammation, cellular recruitment, and bone resorption [29]. Such studies suggest certain candidate genes that may play a central role in the pathogenesis of either THA aseptic failure or THA deep infection.

In this study, we used a candidate gene case-control methodology to determine if well-described functional variation (polymorphism) within the MBL gene is associated with aseptic or septic failure of THA owing to its role in innate immunity. It has a role in the opsonization of bacteria by binding to surface mannose and amino-acetylglucosamine residues on pathogen cell surfaces. It also affects complement activation by an interaction with MBL-associated serum proteinases.

We demonstrated statistically significant associations between the promoter −550 SNP and codon 54 SNP and THA failure. The significant promoter SNP C allele leads to decreased MBL gene tran-

scription. The codon 54 SNP codes for a single amino-acid change in the collagen-like part of the MBL molecule. Both lead to decreased levels of circulating MBL. As such, it is possible that MBL gene variation may predispose to deep bacterial infection in THA, as it has been suggested to be in other infectious diseases [15].

Most in vivo and tissue retrieval studies on failed THAs suggest a chronic, immunologically mediated inflammation reaction. One possible antigenic stimulus is protein-coated particulate wear debris leading to the activation of macrophages and lymphocytes. No study has demonstrated a role for acute inflammation in the development of aseptic loosening [30], although in vitro complement activation by polyethylene wear debris has been shown [31]. Mannose-binding lectin acts like the complement component C1q and, in addition to activating the complement cascade through the lectin pathway, is also very effective in the removal of immune complexes. A lack of MBL may mean less activation of the complement system or humoral response to opsonins and direct the host response more strongly to a cellular immunologic mechanism [32]. Interestingly, patients with rheumatoid arthritis have elevated levels of complement components and autoantibodies but have clearly been shown to have improved survival of THA as compared with patients with osteoarthritis [33].

Association studies such as the one described in this report can be very useful in elucidating the relationship between a 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 managed to collect a large number of study patients and control subjects, with approximately twice as many control subjects as study patients in the aseptic group. Slightly fewer septic case patients were recruited, which probably represents the lower prevalence of this complication. We recognize the statistical need to have as large study groups as possible and are continuing to increase our sample size. One difficulty with this is that we have set highly stringent recruitment criteria in an attempt to avoid population stratification. Only patients with the same diagnosis, of the same ethnicity, and who had the same implant and method of implantation were recruited. Clinical and radiologic criteria were used and only patients with early aseptic or septic failure were recruited, as opposed to control subjects who had well-fixed implants beyond what is accepted at 10 years as long-term survival of a THA, to make the distinction between control subjects and study patients very strict.

The discovery of putative genetic effects on the survival of THAs represents a novel method of investigating the basic science behind prosthetic failure and a potential answer to the common scenario of such failure that cannot be explained by mechanical, technical, or environmental factors. Of course, any such genetic component is probably complex in nature and dependent on numerous gene pathways. The identification of these new pathogenetic mechanisms of THA failure makes new indicators of disease susceptibility and prognosis plus new drug targets direct possibilities. At present, we are continuing to increase our sample size and investigating other suitable candidate genes. In addition, we aim to start a prospective study that will attempt to ascertain if genotype can be related to phenotype (ie, success or failure of a THA) and possibly develop an algorithmic method of planning patient follow-up, type of implant selected, and age at which surgery is offered.

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