TNF-ALPHA PROMOTER REGION POLYMORPHISMS AND THEIR RELATIONSHIP TO OSTEOLYSIS

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Relevance to Musculoskeletal Conditions: The most common mode of failure in total joint arthroplasty today is aseptic loosening secondary to wear debris induced periprosthetic osteolysis. The identification of genetic risk factors associated with the development of osteolysis, might allow one to be able to predict patients most at risk for developing implant loosening.

Introduction: Clinically it has been observed that some patients with total hip arthroplasty develop radiographic evidence of excessive polyethylene wear and yet have little or no osteolysis. Conversely, other patients have little or no polyethylene wear radiographically and yet they develop severe and progressive osteolysis. It would seem that host factors account for the disparate observations and the variability in response. TNF-α has been identified as one of the important cellular mediators in the development of osteolysis. Kwiatkowski et al. was the first to show an association between a specific TNF-α promoter region polymorphism at the –308 bp position and susceptibility to cerebral malaria.1 The two alleles, TNF1 and TNF2 are identical except for a guanine to adenine substitution at –308 bp position. TNF2 has been associated with increased TNF-α production. From these observations we hypothesized that TNF-α promoter region polymorphisms may have an association with the development of osteolysis in patients with total hip arthroplasty.

Materials and Methods:

Patient Cohorts: The radiographs and medical records of patients having undergone total hip replacement at our institution by a single surgeon were randomly reviewed. The first 72 patients meeting the inclusion criteria for either Group I (Osteolysis) or Group II (No Osteolysis) were contacted and asked to donate 20 cc of peripheral blood. Inclusion criteria for Group I included radiographic evidence of osteolysis in Gruen zones II – VI, measuring > 1 cm with less than 10 year follow-up. Inclusion criteria for Group II included no evidence of osteolysis in Gruen zones II – VI with greater than 10 year follow-up. Thirty-two patients, (15 Group I, 17 Group II) agreed to participate and comprise the study cohort.

TNF-α Gene Typing: Genomic DNA was extracted from the peripheral blood and 100 ng was added to a 25 μl reaction mixture containing 1μl of each primer (5’-CACAACACAG-GCCTCAGGACTC-3’ and 5’-AGGGAGGCTCAGGTTG-CTG-3’) with 100 μM of each dNTP, 67 mM Tris-HCl, 16mM (NH4)2SO4, 2 mM MgCl2, and 0.01% Tween-20. After heating at 95°C for 1 min, 1.5 U of Taq DNA polymerase was added, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, then a final 10 min at 72°C. The resultant PCR product was purified and then directly sequenced using the sense primer and fluorescence labelled nucleotides. Template-free controls were used in each experiment.

For the allele specific dot blot assay, the PCR product was denatured with 0.4M NaOH, transferred onto a nylon membrane via a vacuum manifold, and fixed for 1 min in UV light. Allele-specific oligonucleotides (5’-AGGGGACATGGGACCGG-3’ for the TNF1 allele and 5’-AGGGG-CATGAGGACCGG-3’ for the TNF2 allele), end-labeled with digoxigenin-ddUTP (Boehringer Mannheim), were hybridized to the membranes in 3 M TMAC (tetrathymethylammonium chloride) solution at 55°C for 1 h. Excess probe was removed by washing in 2 x SSPE/0.1% SDS at room temperature and subsequently in 3 M TMAC at 58°C. The membranes were washed with antidigoxigenin Fab fragments and, after washing to remove excess antibody, they were incubated with Lumigen PPD (Boehringer Mannheim) and exposed to X-ray film for 15-30 minutes. Allele type was scored by two independent observers. The accuracy of this method was confirmed by DNA sequencing of the PCR product.

Statistical analysis was performed using the Chi-Square Test for contingency tables.

Results: Gene typing results revealed that of the fifteen patients with osteolysis (Group I), there were 12 homozygotes for the TNF1 allele (See Fig. 2) and 3 heterozygotes (See Fig. 1). Of the seventeen patients without osteolysis (Group II), there were 13 homozygotes for the TNF1 allele and 4 heterozygotes. There were no homozygote TNF2 alleles in either group. There was no statistical significance between the two groups to suggest a correlation between development of osteolysis and presence of the TNF2 allele or its heterozygote.

Discussion: Based on Kwiatkowskis’ original study, one might expect patients with osteolysis to have a 7 to 8 fold increased incidence of the TNF2 allele compared to patients without osteolysis.1 However, the present study failed to show an association between the presence of the TNF2 allele and the development of periprosthetic osteolysis. Several explanations may account for this observation. One may be that a relationship between the TNF2 allele and the development of osteolysis does not exist. Another may be that the number of patients did not allow us to demonstrate a difference. Yet another explanation may be the seemingly unavoidable problems associated with defining an appropriate cohort. Although attempts were made to limit the influence of other factors such as surgical technique, implant design, and other related biomechanical issues, these factors cannot be completely excluded from any study cohort. Despite the limitations of this study, we think the approach of trying to identify genetic markers or host risk factors that might predict the clinical outcome in total joint arthroplasty is worthy of further effort.

Conclusion: The variability in host response to particulate wear debris does not seem to be related to the presence or absence of the TNF2 allele in the promoter region of the TNF-α gene. Further investigation, however, is warranted and will hopefully reveal one or more likely several polymorphisms with an association to periprosthetic osteolysis.

References: