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Single nucleotide polymorphisms in the interleukin-6 gene promoter, tumor necrosis factor-α gene promoter, and transforming growth factor-β1 gene signal sequence as predictors of time to onset of aseptic loosening after total hip arthroplasty: preliminary study

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Abstract

Background. Aseptic loosening resulting from inflammatory response to the implant wear debris is the major cause of late total hip arthroplasty (THA) failure. We examined single nucleotide polymorphisms in genes encoding for three involved cytokines — interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and transforming growth factor-β1 (TGF-β1) — as potential predictors of time to onset of aseptic instability.

Methods. A total of 41 patients/45 total hip endoprostheses (same type, same surgeon) were followed up for as long as 18 years. They were genotyped for the *IL-6* promoter (–597G→A) and (–572G→C), *TNF-α* promoter (–308G→A), and *TGF-β1* signal sequence ([29]T→C) transitions. Cox regression was performed on the prosthesis survival.

Results. Overall, 22 of 45 prostheses developed aseptic instability. Cumulative survivals at 10 and 15 years after THA were 95.6% and 66.6%, respectively. The effect of a particular polymorphic site was estimated with adjustment for sex, age at THA, reason for THA, and the effects of other analyzed sites. The hazard ratio (HR) for genotype T/T versus "C-allele carriage" at the *TGF-β1* site was 8.23 [95% confidence interval (CI) 1.45–46.8] (P = 0.017) or 5.70 (1.39–23.4) (P = 0.016) when the *IL-6* promoter sites were considered as a "combination of genotypes (–597) | (–572)." The most prevalent combination of genotypes at *IL-6* sites was G/A (–597) | C/C (–572). HR for this combination (versus other combinations) was 5.43 (1.73–17.0) (P = 0.004) when "*TGF-β1* ([29]T→C)" was considered as a three-level factor (three possible genotypes), and 4.92 (1.71–14.1) (P = 0.003) when *TGF-β1* site was considered as a two-level factor (T/T and "C-allele carriage"). The HR for the "A-allele carriage" at *TNF-α* (–308G→A) could not be determined (only two patients had the G/G genotype).

Conclusions. This preliminary study is the first to suggest that the *TGF-β1* signal sequence ([29]T→C) and *IL-6* promoter (–597G→A) | (–572G→C) transitions are predictive for the time to onset of aseptic instability after THA.

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Introduction

Aseptic loosening is the major cause of late hip endoprosthesis failure. It results from an aseptic inflammatory reaction induced by the implant wear debris accumulating at the prosthesis interface and is mediated by numerous cellular and humoral factors.[1] It may affect the acetabular cup, the femoral stem, or both elements.[1–3] Aseptic loosening is more likely to occur earlier after arthroplasty with certain types of prosthetic devices/materials (e.g., with Endler polyethylene cups or cemented and smooth-threaded uncemented cups when compared to coated uncemented cups and with cemented or uncoated/uncemented femoral stems when compared to coated uncemented stems).[1–4] Also, aseptic loosening is likely to occur earlier with less experienced surgeons.[5,6] Patients requiring hip arthroplasty because of developmental hip dysplasia (DDH) or complications of the femoral neck fracture are likely to develop aseptic loosening earlier than patients with primary osteoarthritis.[7] High body mass index (BMI ≥ 30) appears to favor earlier onset of aseptic loosening after arthroplasty used to treat complications of femoral neck fractures.[8] In other situations, BMI at the time of surgery seems to be irrelevant.[9,10] Patients < 55 years of age at the time of arthroplasty[7] and particularly patients < 46 years of age[2] are likely to develop aseptic loosening earlier than older patients. Aseptic loosening appears to occur earlier in men than in women.[9] Overall, however, these factors explain only some of the variability in timing of aseptic loosening after total hip arthroplasty (THA), which suggests a role for "individual susceptibility" to this complication determined by factors other than demographic or morbidity characteristics.[1]

Recently, Wilkinson and co-workers reported the association of a single nucleotide polymorphism (SNP) in the promoter region of the tumor necrosis factor-α (TNF-α) gene (–238G→A) and the occurrence of aseptic loosening: The odds of carrying the less frequent "A

allele" were greater in patients who had experienced aseptic loosening than in patients who had not [odds ratio (OR) 1.8, 95% confidence interval (CI) 1.0–3.2].[10] They suggested that the genetic factors might help explain the variability in aseptic loosening occurrence, and genes encoding for the mediators of aseptic loosening appeared to be good candidate genes.[10] Following this logic, the current study is a preliminary investigation of further potential genetic "contributors" to this late complication of THA; SNPs in genes encoding for transforming growth factor-β1 (TGF-β1), interleukin 6 (IL-6), and TNF-α. TGF-β1, IL-6, and TNF-α are important mediators of aseptic inflammation resulting in THA instability.[1]

TGF-β1 is located on chromosome 19 (q13.1–13.3).[11] Polymorphisms described in this gene include, among others, $^{29}T \rightarrow C$ transition in the signal sequence.[12] At least in middle-aged European women, carriage of the "C-allele" in this position is associated with lower circulating levels of TGF-β1.[13]

IL-6 is located on chromosome 7 (p21)[14] and has several polymorphic sites, including (–597G→A) and (–572G→C) in the promoter region. These sites are involved in regulation of IL-6 production through complex interactions with other polymorphisms in the *IL-6* promoter.[15,16]

TNF-α is located on chromosome 6 (p21.1–21.3) in the human leukocyte antigen complex. One of the polymorphic sites is (–308G→A) in the promoter region.[17] Homozygous genotype A/A at this site is associated with higher levels of circulating TNF-α and increased susceptibility to a number of inflammatory diseases.[18]

In the present study, these SNPs are evaluated as potential predictors of time to onset of aseptic instability after THA.

Patients and methods

Patients

The study was approved by the local Ethics Committee of Zagreb University School of Medicine Clinical Center.

We retrospectively identified a cohort of 72 patients bearing 80 total hip prostheses who met the following predefined criteria: (1) at least 12 years had elapsed since the surgery; (2) prostheses were of the same type (Endler polyethylene uncemented actabular cup, Zweymueller uncemented femoral stem) and were implanted by the same surgeon; (3) they had been followed up on a regular basis (time intervals of around 12 months, if not shorter due to subjective difficulties); (4) patients consented to a control visit in July 2003 ("final visit"); and (5) prosthesis failure (if it had occurred) was due to aseptic loosening.[19] Altogether, 41 of these patients bearing a total of 45 prostheses gave written informed consent for DNA analysis, and this is the subset included in the current report. All of the 41 subjects were Caucasian Croatian residents, with most residing within the broader Zagreb area, although some were referred to our insitution from various other parts of Croatia (patients 25 and 16, respectively).

Methods

For the hip replacement procedure, we always we used a modified anterolateral approach (Watson-Jones) and placed the femoral stem in the neutral position. The follow-up visits included clinical examination and analysis of anteroposterior and lateral X-ray views according to DeLee and Charnley[20]: The acetabular cup contact surface area and the stem area were divided in three and seven zones, respectively, in each view and were inspected for the presence of radiolucencies, osteolysis, and cup or stem migration.

The endpoint in this study was prosthesis failure due to aseptic loosening (clinical and/or radiological criteria for revision were met). We applied Krugluger and Eyb's criteria for radiological failure[21]: (1) level 1 — stable (visible threads or a radiolucent line of ≤1 mm in width in no more than a single area); (2) level 2 — early instability (visible threads or radiolucent lines of 1–2 mm in two areas); (3) level 3 — probable instability (visible threads or radiolucent lines of 1–2 mm in width in two or more areas; osteolytic defect of >2 mm); and (4) level 4 — definite instability (visible threads or radiolucent lines of 1–2 mm in width in several areas; osteolytic defect >2 mm; endoprosthesis migration). Level 2 radiological findings (acetabulum or stem) were considered a prosthesis failure if combined with clinical symptoms. Level 3 or 4 findings were considered a failure regardless of the clinical symptoms.

Genomic DNA was extracted by proteinase K digestion followed by phenol extraction and ethanol precipitation of the peripheral venous white blood cells. The isolated DNA samples were quantified and subjected to a polymerase chain reaction (PCR). The SNP analysis in *TGF-β1* was done by a sequence-specific PCR based on mismatched 3′ nucleotide in the sense primer.[13] The amplicons were run through a 2% agarose gel stained with ethidium-bromide (0.5 μg/ml). The SNPs in *IL-6* and *TNF-a* were investigated by PCR-restriction fragment length polymorphism (RFLP) analysis using the restriction endonucleases *Fok* I, *BsrB* I *(IL*-6), and *Nco* I (*TNF-α*) (New England BioLabs, Beverly, MA, USA) as described elsewhere.[16,22] Aliquots of the PCR products (6–12 μl, depending on the amount of the product) were digested at 37°C for 24 h. The fragments were separated in 10% nondenaturing polyacrylamide gel

Table 1. Primers, cycle conditions, fragment lengths, polymorphism position, enzymes and digested fragments determining the PCR and RFLP methods

Gene	Primers	No. of cycles/annealing temperature	Fragment length (bp)	Polymorphism	Enzymes	Digested fragment length	Ref.
IL-6	Sense primer 5′-GCA ACT TTG AGT GTG TCA CG-3′	35 cycles/at 57°C	169	–597A→C	*Fok* 1	122/47	16
	Antisense primer 5′-TGA CGT GAT GGA TGC AAC AC-3′	35 cycles/at 57°C	163	–572G→C	*Bsr*B 1	101/62	16
TGF-β1	Sense primer 1 5′-CTC CGG GCT GCG GCT GCT GCT-3′	40 cycles/at 62°C	346	T^{29}→C			13
	Sense primer 2 5′-CTC CGG GCT GCG GCT GCT GCC-3′	40 cycles/at 62°C					
	Antisense primer 5′-GTT GTG GGT TTC CAC CAT TAG-3′	40 cycles/at 62°C					
TNF-α	Sense primer 5′-AGG CAA TAG GTT TTG AGG GCC AT-3′	35 cycles/at 57°C	107	–308G→A	*Nco* 1	42/65	22
	Antisense primer 5′-TCC TCC CTG CTC CGA TTC CG-3′	35 cycles/at 57°C					

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

and silver-stained. The primers used in these reactions are shown in Table 1. For all reactions, 250 ng of genomic DNA was used as a template, and the reactions were run in Applied Biosystems GeneAmp PCR System 2400. The reaction mixture (25 µl) contained dNTPs (50 µM each), 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 8 pmol of each primer, and 0.25 U of rTaq polymerase (TaKaRa, Shiga, Japan). The reaction conditions are shown in Table 1.

Statistical analysis

Each prosthesis was treated as an independent case.[23] The time to the event was determined as the time (years) elapsed since THA until a revision was indicated or until the final visit, whichever occurred first. Prostheses that presented with aseptic loosening during the follow-up or at the "final visit" were "failures"; and prostheses that were stable at the final visit were "censored data." Proportional hazard regression was performed on the prosthesis survivorship data by applying *proc tphreg* in the SAS system for Windows version 9.1 (SAS, Cary, NC, USA).

Four models were analyzed. The first model (model 1) included the following independents: age at the time of surgery (dichotomized as <46 years and ≥46 years),[2] sex (male or female), underlying diseases (DDH or "other"), and the analyzed polymorphic sites — *IL-6* promoter (–597G→A) and (–572G→C), *TNF-α* promoter (–308G→A), and *TGF-β1* signal sequence (^{29}T→C) — each with three levels (three possible genotypes). The second model (model 2) differed from model 1 in that factor "*TGF-β1* signal sequence (^{29}T→C)" had two levels: T/T genotype or genotypes with "C allele" (T/C or C/C — i.e., "C allele carriage").[13] The reference genotypes at the investigated sites were the genotypes that prevailed in the analyzed sample. The third model (model 3) differed from model 1 in that factors "*IL-6* promoter (–597G→A)" and "*IL-6* promoter (–572G→C)" were substituted with a single factor: "combination of genotypes at *IL-6* promoter –597 and –572 polymorphic sites." This factor was planned to have two levels: the most frequent (–597) | (–572) combination in the analyzed sample versus "other combinations." The last model (model 4) was planned to be a combination of models 2 and 3. Considering the literature data[9,10] and the fact that only three patients had a BMI at the time of surgery of >30, BMI was not included in the analyzed models.

Results

General characteristics of the analyzed patients/hips are summarized in Table 2. Only two patients were older

Table 2. General characteristics of the analyzed patients and hips

No. of patients, sex (M/F)	41 (13/28)
No. of hips, sex (M/F)	45 (15/30)
Age (years) at the time of surgery (by hip)	44 (26–58)
Distribution by age at the time of surgery (by hip)	
<46 Years	27
≥46 Years	18
BMI at the time of surgery (by hip)	25 (20–34)
Causes leading to hip arthroplasty (by hip)	
Developmental dysplasia of the hip (DDH)	31
Idiopathic aseptic necrosis of the femoral head	6
Trauma (fracture, epiphysiolysis)	4
Mb Bechterew	4
Follow-up period (years) (by hip)	15 (5–18)
Prostheses with aseptic loosening	22
Isolated aseptic loosening of the cup	15
Aseptic loosening of the cup and the stem	7

Data are counts or the median and range
BMI, body mass index

Table 3. Life table for the 45 analyzed endoprotheses

Years since surgery	At risk (no.)	Failed (no.)	Censored (no.)	Cumulative survival	Cumulative hazard (95% CI)
1	45	0	0	1	0
2	45	0	0	1	0
3	45	0	0	1	0
4	45	0	0	1	0
5	45	0	0	1	0
6	45	1	0	1	0.022 (0.003–0.158)
7	44	0	0	0.978	0.022 (0.003–0.158)
8	44	1	0	0.978	0.045 (0.011–0.180)
9	43	0	0	0.956	0.045 (0.011–0.180)
10	43	2	0	0.956	0.091 (0.034–0.244)
11	41	3	0	0.911	0.165 (0.078–0.345)
12	38	1	0	0.844	0.191 (0.095–0.382)
13	37	4	0	0.822	0.299 (0.170–0.527)
14	33	3	1	0.733	0.391 (0.235–0.651)
15	29	1	5	0.666	0.429 (0.262–0.704)
16	23	2	5	0.641	0.527 (0.326–0.850)
17	16	1	2	0.578	0.593 (0.368–0.957)
18	13	1	8	0.539	0.704 (0.424–1.170)
18+	4	2	2	0.480	1.371 (0.666–2.842)

Time since arthroplasty is given as 1-year intervals in line with the frequency of the follow-up visits
Failed, prostheses presenting with aseptic loosening; censored, "final visit" performed at the particular time since arthroplasty, no signs of aseptic loosening

than 55 years at the time of THA (57 and 58 years, respectively), and only three had a BMI > 30 at the time of surgery (31, 33, and 34, respectively). Arthroplasty was mainly due to DDH. The median follow-up period was 15 years with 22 of 45 prostheses developing aseptic loosening (event rate 48.9%). Owing to the small sample size, certain genotypes were observed in only a few patients: five patients (five prostheses) had a T/T genotype at position 29 in the *TGF-β1* signal sequence; two subjects (two prostheses) had a G/G genotype at position –308 in the *TNF-α* promoter; three subjects (four prostheses) had a G/G genotype and six subjects (six prostheses) a G/C genotype at position –572 in the *IL-6* promoter (Fig. 1). Survivorship data for the entire cohort are summarized in the life table (Table 3). Figure 1 illustrates the development of cumulative hazards of aseptic loosening by genotype.

Prosthesis survivorship was analyzed in four regression models that differed in the number of levels per particular independent variable-polymorphic site (Table 4). "Larger" models (models 1 and 2) were borderline significant ($0.05 < P \leq 0.1$), and "smaller" models (models 3 and 4) were statistically significant ($P < 0.05$) (Table 4). The hazard ratio for age at THA <

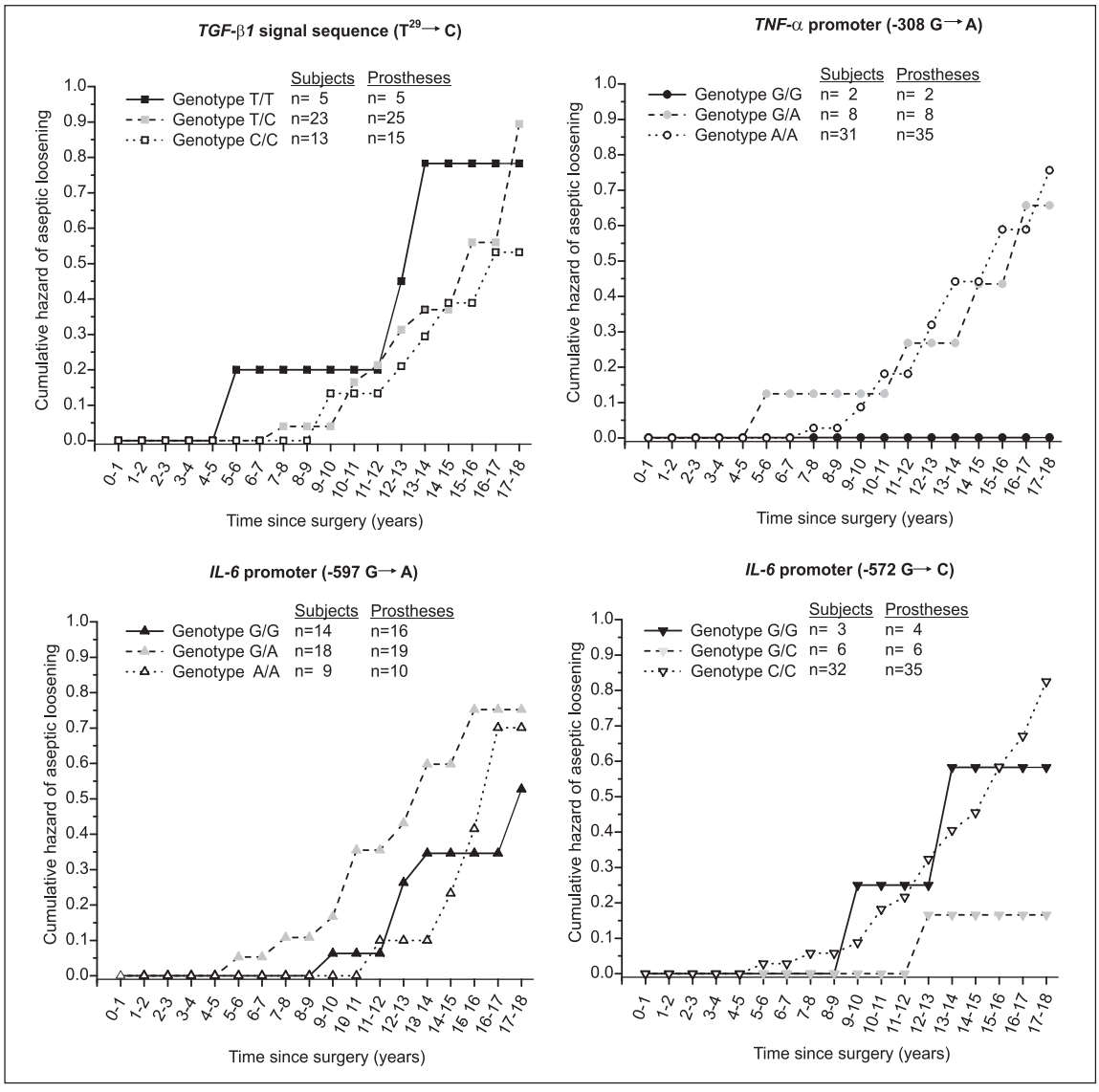

Fig. 1. Prevalence of genotypes at the investigated sites and cumulative hazard of aseptic loosening over time after total hip arthroplasty, by genotype. Cumulative hazard is from the life-table analysis

46 years (vs. ≥46 years) was consistently around 2.0–2.5 across all models and was, within the analyzed sample, not statistically significant (models 1 and 2) or was borderline significant ($0.05 < P \leq 0.1$) (models 3 and 4) (Table 4, Fig. 2). Hazard ratios for female sex and reasons for THA other than DDH (vs. DDH) were consistently around 0.5–0.6 and were not statistically significant within the available sample (Table 4, Fig. 2).

With adjustment for age, sex, reason for THA, and other investigated polymorphic sites, the main effect of factor "*TGF-β1* signal sequence (^{29}T→C)" was statistically significant across all models (Table 4). The most prevalent genotype at this site within the analyzed sample was T/C (13 subjects, 15 prostheses) (Fig. 1). When "*TGF-β1* signal sequence (^{29}T→C)" was considered as a three-level factor (models 1 and 3), hazard ratios for genotype T/T (vs. T/C) were around 9.0 and around 6.5 in the two models, respectively ($P < 0.05$) (Fig. 2). In models 2 and 4, "*TGF-β1* signal sequence (^{29}T→C)" was considered as a two-level factor — i.e., as T/T (five subjects, 5 prostheses) vs. "C-allele carriage" (36 subjects, 40 prostheses) (Fig. 1). Hazard ratios for genotype T/T were around 8.2 and around 5.7 in the two models, respectively ($P < 0.05$) (Fig. 2).

The fact that there were only two subjects (two prostheses) with the genotype G/G at the –308 (G→A) site in the *TNF-α* promoter disabled a meaningful analysis of the potential effect of this polymorphism on the prosthesis survival.

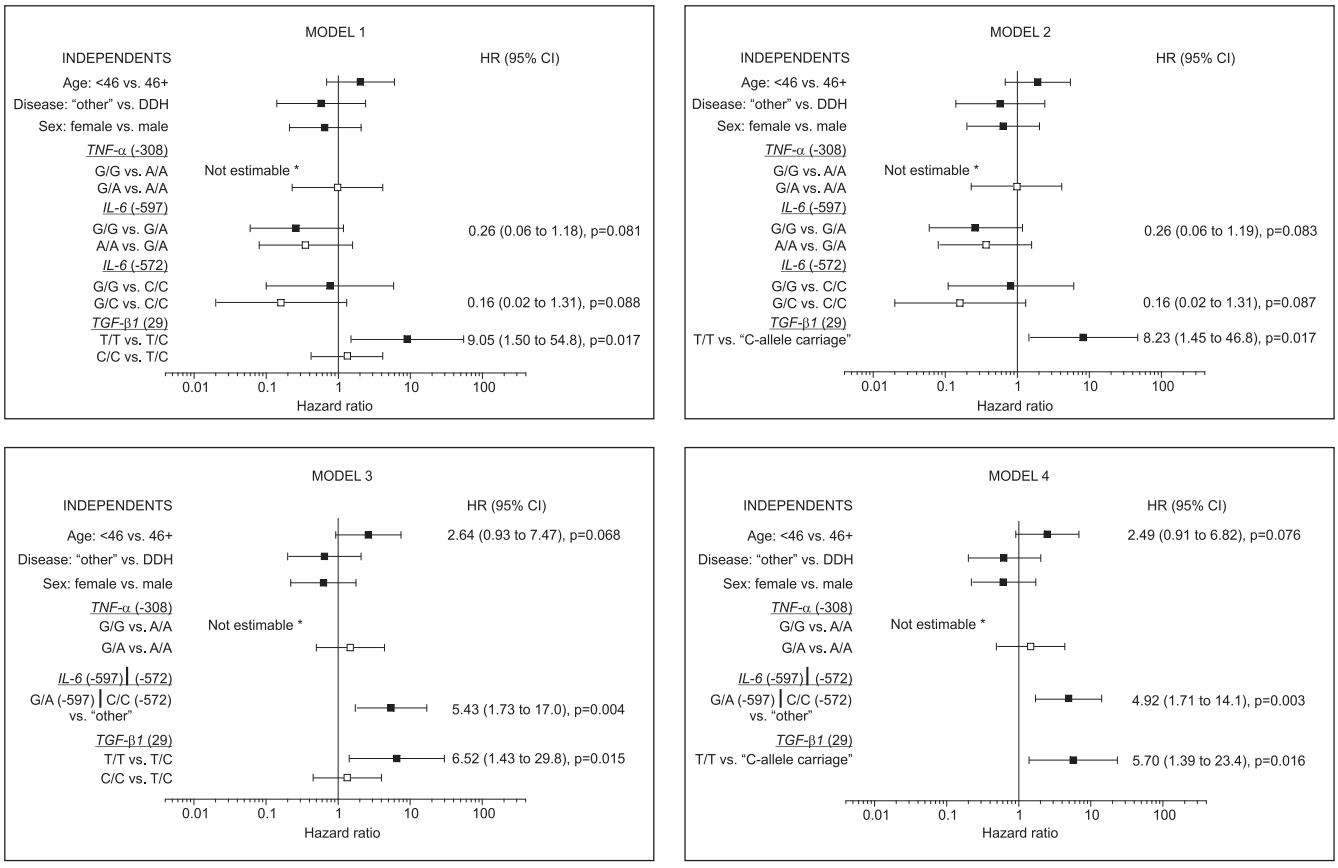

Fig. 2. Hazard ratios (HRs) from the proportional hazard regression analysis of time to onset of aseptic loosening after total hip arthroplasty in the four models depicted in Table 4. For a particular factor — the polymorphic site — the HR was determined in respect to the most prevalent genotype in the analyzed sample. When factor "*TGF-β1* (^{29}T→C)" had two levels (models 2 and 4), HR was determined for genotype T/T in respect to "C-allele carriage." When the factor "combination of genotypes *IL-6* (–597) | (–572)" with two levels was included in the analysis (models 3 and 4), the HR was determined for the combination G/A (–597) | C/C (–572), which was the most frequent one in the analyzed sample in respect to "other combinations." Statistically significant or borderline significant HRs are presented graphically and numerically. *G/G genotype at this site was found for only two hips. Both were followed up for 17 years and did not develop aseptic loosening ("censored")

Table 4. Summary of proportional hazard regressions on time to onset of aseptic instability after total hip arthroplasty for four analyzed models

Model 1 LR test df 11, $p = 0.082$			Model 2 LR test df 10, $p = 0.058$			Model 3 LR test df 8, $p = 0.038$			Model 4 LR test df 7, $p = 0.022$		
Effects	df	*P*	Effects	df	*P*	Effects	df	*P*	Effects	df	*P*
Age	1	0.199	Age	1	0.220	Age	1	0.068	Age	1	0.076
Disease	1	0.448	Disease	1	0.453	Disease	1	0.467	Disease	1	0.432
Sex	1	0.469	Sex	1	0.450	Sex	1	0.381	Sex	1	0.352
IL-6 (–597)	2	0.143	*IL-6* (–597)	2	0.142	IL-6 (–597 | –572)	1	0.004	IL-6 (–597 | –572)	1	0.003
IL-6 (–572)	2	0.233	*IL-6* (–572)	2	0.228	*TGF-β1* (29)	2	0.049	*TGF-β1* (29)	1	0.016
TGF-β1 (29)	2	0.055	*TGF-β1* (29)	1	0.017	*TNF-α* (–308)	2	0.781	*TNF-α* (–308)	2	0.792
TNF-α (–308)	2	0.999	*TNF-α* (–308)	2	0.999						

The likelihood ratio (LR) test was used to assess the significance of a model. Type III tests based on Wald statistics assessed the effects of individual factors ("main effects"). The factors age and disease were dichotomized (see Patients and methods). In model 1, each factor-polymorphic site had three levels (three possible genotypes). In model 2, factor *TGFβ1* (29) had two levels: "T/T" or "C-allele carriage." In model 3, factors *IL-6* (–597) and *IL-6* (–572) were replaced by a single factor, a combination of genotypes at (–597) and (–572), with two levels: "G/A (–597) | C/C (–572)", which was the most frequent combination in the analyzed sample or "other." Model 4 was a combination of models 2 and 3

In models 1 and 2, "*IL-6* promoter (–597G→A)" and "*IL-6* promoter (–572G→C)" were considered as separate three-level factors, and their main effects were insignificant (Table 4). The most frequent genotypes in the analyzed sample were G/A and C/C at –597 and –572, respectively (Fig. 1). Hazard ratios determined in respect to these reference genotypes showed borderline significance ($0.05 < P \leq 0.1$) for G/G at –597 (point estimate 0.26) and for G/C at –572 (point estimate 0.16) (Fig. 2). We observed seven of nine possible combinations of genotypes at *IL-6* promoter (–597) and (–572). The most prevalent one was G/A (–597) | C/C (–572) (17 subjects, 17 prostheses). Among 21 patients bearing 22 prostheses that developed aseptic loosening during the follow-up period, 10 subjects (10 prostheses) had this genotype. On the other hand, among 20 subjects bearing 23 prostheses that did not develop aseptic loosening during the follow-up period, 7 subjects (7 prostheses) had this genotype. In models 3 and 4, factors "*IL-6* promoter (–597)" and "*IL-6* promoter (–572)" were replaced with a factor "combination of genotypes *IL-6* (–597) | (–572)" with two levels: G/A –597 | C/C –572 or "other." Both models were significant, and the main effect of this factor was significant ($P < 0.05$, respectively) (Table 4). The hazard ratio for the combination G/A –597 | C/C –572 versus "other" was around 5.4 (model 3) and around 4.9 (model 4) ($P < 0.05$, respectively) (Fig. 2).

Discussion

Individual susceptibility to aseptic loosening after THA determined by patient-related factors other than demographic or morbidity characteristics has been recognized.[1] Recently, carriage of "A allele" at polymorphic site (–238G→A) in the *TNF-α* promoter was shown to be associated with higher odds of aseptic loosening.[10] This was the first documentation of the genetic contribution to this late complication of THA.[10] The genetic component in this condition is likely to include modest contributions by many polymorphisms, and genes encoding for cytokines involved in the development of aseptic instability appear to be good candidate genes.[10] Following this logic, the current report addressed several further SNPs, including *TGF-β1* signal sequence (^{29}T→C), *IL-6* promoter (–597G→A) and (–572G→C), and *TNF-α* promoter (–308G→A). Unlike association studies that evaluate the presence of a certain allele or a genotype and the presence of a disease,[24] we investigated these sites as potential predictors of time to onset of aseptic loosening after THA. Each SNP (site) was assessed with adjustment for the effects of other SNPs and with adjustment for sex, age at surgery, and reason for THA.[2,7,9] Because the analyzed group of patients was relatively young, the "cutoff" point for factor "age at THA" was set at 46 years[2] rather than at 55 years.[7] Potential influences of surgeon's skill and prosthetic materials on the outcome variable were controlled for by the fact that all prostheses were of the same type and implanted by the same experienced surgeon (>300 THA procedures by 1985 when the first of the prostheses included in this analysis was implanted). We used standard methodology for detecting aseptic loosening over a regular and long follow-up period (shortest follow-up for a censored observation 13 years) and well-characterized genotyping methodology. As the study was conceived as an exploratory one ("proof of the concept"), the outcome variable was analyzed in four alternative but predefined models that differed in the number of "levels" (degrees of freedom) for factors "*TGF-β1* (^{29}T→C)," "*IL-6* (–597G→A)," and "*IL-6* (–572G→C)." Repeated analysis was therefore not considered as a source of a multiplicity problem.

The *TGF-β1* signal sequence (^{29}T→C) transition results in Leu-Pro substitution at position 10 in the TGF-β1 molecule and affects the peptide export efficiency.[11,12] Genotype T/C appears to prevail in middle-aged European women.[13] In this population, genotype T/T is associated with higher levels of circulating TGF-β1 than the T/C or C/C genotype ("C-allele carriage").[13] Genotype T/C prevailed in our sample as well. Current data are the first to suggest that the genotype at this site is predictive for time to onset of aseptic instability after THA: The hazard ratio for the T/T genotype vs. the T/C genotype or vs. the "C-allele carriage" was consistently significantly ($P < 0.05$) greater than 1, suggesting a higher risk of developing aseptic loosening associated with the T/T genotype. Obviously, the present study is limited by the small sample and the fact that there were only five patients (five prostheses) with the T/T genotype. However, considering the fact that the analyzed sample was a random one (consecutive consenting patients), the fact that we used standard genotyping and radiological/clinical follow-up methodology, and the fact that we controlled for a number of potentially confounding factors (inclusion/exclusion criteria, covariates), we believe that the current observation has a fair level of internal validity. We provide no clues about the functional relation between this SNP and the occurrence of aseptic loosening. It seems, however, that the apparent beneficial effect of higher TGF-β1 levels (and T/T genotype) on bone mineral density seen in "non-THA patients"[13] might be overridden by some counteracting effect in the case of aseptic inflammation resulting in loosening of the hip replacement.

In contrast to a clear-cut association between the *TNF-α* promoter (–238G→A) SNP and aseptic loosening, only a "weak trend" of association between aseptic loosening and *TNF-α* promoter (–308G→A) transition

was reported.[10] Due to the fact that there were only two subjects with the G/G genotype at this site in the current sample, we were unable to evaluate potential effects of this SNP on prosthesis survival after THA.

Several SNPs in the *IL-6* promoter (–597G→A, –572G→C, $-373A_nT_n$, and –174G→C) have been studied for their association with various diseases. Functional studies have demonstrated complex interactions among these sites in the regulation of *IL-6* transcription.[15] Complete linkage disequilibrium between (–597G→A) and (–174G→C) sites[16,25–28] and no disequilibrium[16,25,26,28] or complete negative disequilibrium[27] between (–597G→A) and (–572G→C) sites have been reported in European populations. Studies in Caucasian European populations[15,28] indicate that the –597/–572 haplotypes GG (around 50%) and AG (around 40%) are the most prevalent ones. The most prevalent genotype combinations appear to be G/A –597 | G/G –572 (around 40%) and G/G –597 | G/G –572 (around 30%).[15,18] The fact that 17/41 subjects in the current sample had the combination G/A –597 | C/C –572 (which has not been reported in "control" European populations)[15] and that only 4 of 41 subjects had the G/G | G/G combination and none had the G/A | G/G combination indicates a difference between THA patients and the "general Caucasian European" population. Considering that most of our patients suffered from DDH, this may indicate an association between IL-6 promoter SNPs and DDH. Combination of genotypes G/G | G/G | G/G at –597, –572, and –174 appears to be predictive for better kidney allograft survival: It is associated with lower hazard of allograft rejection than other combinations taken cumulatively.[28] In the current report, individual influences of the *IL-6* (–597) and (–572) sites/genotypes did not appear to have a relevant effect on the prosthesis survival. A similar "lack of the effect" of the –597 or –572 genotype on kidney allograft survival has been reported.[15] However, the G/A | C/C combination of genotypes at these two sites was associated with a markedly increased risk of aseptic loosening (versus other combinations cumulatively) (DDH included as a covariate). Hence, the current data are the first to suggest that SNPs/genotypes in the *IL-6* promoter might be predictive for the time to onset of aseptic instability after THA. Functional links between these SNPs and aseptic loosening await further investigations.

Conclusion

The results of this preliminary study point out the *TGF-β1* signal sequence (^{29}T→C) and *IL-6* promoter (–597G→A) and (–572G→C) transitions as predictive for time to onset of aseptic instability after THA. This suggests that these polymorphic sites deserve further investigation in larger studies that would enable conclusions on prevalence of particular genotypes or alleles among THA patients and their clinical relevance.

References

1. Archibeck MJ, Jacobs JJ, Roebuck K, Glant TT. The basic science of periprosthetic osteolysis. Instr Course Lect 2001;50:185–95.
2. Havelin LI, Vollset SE, Engesaeter LB. Revision for aseptic loosening of uncemented cups in 4,352 primary total hip prostheses: a report from the Norwegian Arthroplasty Register. Acta Orthop Scand 1995;66:494–500.
3. Havelin LI, Espehaug B, Vollset SE, Engesaeter LB. Early aseptic loosening of uncemented femoral components in primary total hip replacement: a review based on the Norwegian Arthroplasty Register. J Bone Joint Surg Br 1995;77:11–17.
4. Eskelinen A, Remes V, Helenius I, Pulkkinen P, Nevalainen J, Paavolainen P. Total hip arthroplasty for primary osteoarthrosis in younger patients in the Finnish Arthroplasty Register: 4,661 primary replacements followed for 0–22 years. Acta Orthop 2005;76:28–41.
5. Smabrekke A, Espehaug B, Havelin LI, Furnes O. Operating time and survival of primary total hip replacements: an analysis of 34,745 primary cemented and uncemented total hip replacements from local hospitals reported to the Norwegian Arthroplasty Register 1987–2001. Acta Orthop Scand 2004;75:524–32.
6. Losina E, Barrett J, Mahomed NN, Baron JA, Katz JN. Early failures of total hip replacement: effect of surgeon volume. Arthritis Rheum 2004;50:1338–43.
7. Furnes O, Lie SA, Espehaug B, Vollset SE, Engesaeter LB, Havelin LI. Hip disease and the prognosis of total hip replacements: a review of 53,698 primary total hip replacements reported to the Norwegian Arthroplasty Register 1987–99. J Bone Joint Surg Br 2001;83:579–86.
8. Mabry TM, Prpa B, Haidukewych GJ, Harmsen WS, Berry DJ. Long-term results of total hip arthroplasty for femoral neck fracture non-union. J Bone Joint Surg Am 2004;86:2263–7.
9. Malchau H, Herberts P, Ahnfelt L. Prognosis of total hip replacement in Sweden: follow-up of 92,675 operations performed 1978–1990. Acta Orthop Scand 1993;64:497–506.
10. Wilkinson JM, Wilson AG, Stockley I, Scott IR, Macdonald DA, Hamer AJ, et al. Variation in the TNF gene promoter and risk of osteolysis after total hip arthroplasty. J Bone Miner Res 2003;18:1995–2001.
11. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, et al. Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. Nature 1985;316:701–5.
12. Derynck R, Rhee L, Chen EY, Van Tilburg A. Intron-exon structure of the human transforming growth factor-beta precursor gene. Nucleic Acids Res 1987;15:3188–9.
13. Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R, Pfeilschifter J. Association of transforming growth factor-beta 1 (TGF beta 1) T29→C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-β1 in a population-based sample of postmenopausal German women. Calcif Tissue Int 2001;69:315–20.
14. Bowcock AM, Kidd JR, Lathrop GM, Daneshvar L, May LT, Ray A, et al. The human interferon-β2/hepatocyte stimulating factor/interleukin-6 gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 1988;3:8–16.
15. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000;275:18138–44.

16. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, et al. Interleukin-6 gene –174G > C and –572G > C promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001;21:1458–63.
17. Udalova IA, Nedospasov SA, Webb GC, Chaplin DD, Turetskaya RL. Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics 1993;16: 180–6.
18. Abraham LJ, Kroeger KM. Impact of the –308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. J Leukoc Biol 1999;66:562–6.
19. Kolundzic R, Sulentic M, Smerdelj M, Orlic D, Trkulja V. Stability of Endler cementless polyethylene acetabular cup: long-term follow-up. Croat Med J 2005;46:261–7.
20. DeLee JG, Charnley J. Radiological demarcation of cemented sockets in total hip replacement. Clin Orthop 1976;121:20–32.
21. Krugluger J, Eyb R. Bone reaction to uncemented threaded polyethylene acetabular components. Int Orthop 1993;17:259–65.
22. Um JY, Park JH, Kim HM. Gene polymorphisms in tumor necrosis factor locus and waist-hip ratio in obese Koreans. Clin Chim Acta 2003;338:117–22.
23. Schwarzer G, Schumacher M, Maurer TB, Ochsner PE. Statistical analysis of failure times in total joint replacement. J Clin Epidemiol 2001;54:997–1003.
24. Gordon D, Finch SJ. Factors affecting statistical power in the detection of genetic association. J Clin Invest 2005;115:1408–18.
25. Villuendas G, San Millan JL, Sancho J, Escobar-Morreale HF. The –597G→A and –174G→C polymorphisms in the promoter of the IL-6 gene are associated with hyperandrogenism. J Clin Endorinol Metab 2002;87:1134–41.
26. Hamid Yh, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, et al. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia 2005;48:251–60.
27. Georges JL, Loukaci V, Poirier O, Evans A, Luc G, Arveiler D, et al. Interleukin-6 gene polymorphisms and susceptibility to myocardial infarction: the ECTIM study. J Mol Med 2001;79:300–5.
28. Muller-Steinhardt M, Fricke L, Muller B, Ebel B, Kirchner H, Harter C. Cooperative influence of the interleukin-6 promoter polymorphisms –597, –572 and –174 on long-term kidney allograft survival. Am J Transplant 2004;4:402–6.