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Source / Izvornik: **Journal of Orthopaedic Science, 2006, 11, 592 - 600**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/doi: 10.1007/s00776-006-1069-y

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:345305

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

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Clinical study (prognostic)

Running title: Cytokine SNPs and hip arthorplasty

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Abstract

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

Methods. Forty one patients/45 total hip endoprostheses (same type, same surgeon) were followed-up for up to 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, 22/45 prostheses developed aseptic instability. Cumulative survival at 10 and 15 years after THA was 95.6% and 66.6%, respectively. 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. Hazard ratio (HR) for genotype T/T vs. "C-allele carriage" at *TGF-β1* site was 8.23 (95% confidence interval 1.45 to 46.8), p=0.017; or 5.70 (1.39 to 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 (vs. other combinations) was 5.43 (1.73 to 17.0), p=0.004 when "*TGF-β1* (^{29}T→C)" was considered as a 3-level factor (3 possible genotypes), and 4.92 (1.71 to 14.1), p=0.003, when *TGF-β1* site was considered as a 2-level factor (T/T and "C-allele carriage"). HR for the "A-allele carriage" at *TNF-α* (-308G→A) could not be determined (only 2 patients had the G/G genotype).

Conclusion: This preliminary study is the first to suggest *TGF-β1* signal sequence (^{29}T→C) and *IL-6* promoter (-597G→A) | (-572G→C) transitions as predictive for time to onset of aseptic instability after THA.

Introduction

Aseptic loosening is the major cause of the late hip endoprosthesis failure. It results from 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 either the acetabular cup or 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, for example with Endler polyethylene cups or cemented and smooth-threaded uncemented cups as opposed to coated uncemented cups, and with cemented or uncoated uncemented femoral stems as opposed to coated uncemented stems[1-4]. Also, aseptic loosening is likely to occur earlier with less experienced surgeons[5,6]. Patients requiring hip arthroplasty due to developmental hip dysplasia (DDH) or complications of the femoral neck fracture are likely to develop aseptic loosening earlier than the 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 the femoral neck fracture[8]. In other situations, BMI at the time of surgery seems to be irrelevant[9,10]. Patients younger than 55 years 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, the mentioned factors explain only a part of 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 association of a single nucleotide polymorphism (SNP) in the promoter region of the tumor necrosis factor alpha (TNF-α) gene (-238 G→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 1.8, 95% CI 1.0 to 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 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 the 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 (–597 G→A) and (–572 G→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) within the human leukocyte antigen complex. One of the polymorphic sites is (–308 G→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 total hip arthroplasty.

Patients and Methods

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

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

For the hip replacement procedure, we always we used modified anterior-lateral approach (Watson-Jones) and placed the femoral stem in the neutral position. The follow-up visits included clinical examination and analysis of anterior-posterior and lateral X-ray views according to DeLee and Charnley[20]: the acetabular cup contact surface area and the stem area were divided in 3 and 7 zones, respectively, in each view, and were inspected for presence of radiolucencies, osteolysis, and cup or stem migration.

The end-point 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]: a) level 1 – stable (visible threads or a radiolucent line of ≤1 mm in width in no more than a single area); b) level 2 – early instability (visible

threads or radiolucent lines of 1-2 mm in two areas); c) 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 d) 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) where 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 ug/ml). The SNPs in *IL-6* and *TNF-a* were investigated by PCR-restriction fragment length polymorphism (RFLP) analysis using restriction endonucleases *Fok* I, *BsrB* I *(IL*-6) and *Nco* I (*TNF-α*) (New England BioLabs) as described elsewhere[16,22]. Aliquots of the PCR products (6-12 uL, depending on the amount of the product), were digested at 37°C for 24 h. The fragments were separated in 10% non-denaturing polyacrylamide gel 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 BiosystemsGeneAmp PCR System 2400. The reaction mixture (25 uL) 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). The reaction conditions are shown in Table 1.

Statistical analysis

Each prosthesis was treated as an independent case[23]. Time to event was determined as time (years) elapsed since THA until indication for revision was made or until the final visit, whichever occurred first. Prostheses presenting 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 SAS system for Windows version 9.1 (SAS Corp. 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 (-597 G→A) and (-572 G→C), *TNF-α* promoter (-308 G→A) and *TGF-β1* signal sequence (^{29}T→C) – each with 3 levels (3 possible genotypes). 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 within the analyzed sample. Third model (Model 3) differed from Model 1 in that factors "*IL-6* promoter (-597 G→A)" and "*IL-6* promoter (-572 G→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 vs. "other combinations". The last model (Model 4) was planned to be a combination of Model 2 and Model 3. Considering the literature data[9,10] and the fact that only 3 patients had BMI at the time of surgery >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 than 55 years at the time of THA (57 and 58 years, respectively) and only 3 had BMI >30 at the time of surgery (31, 33 and 34, respectively). Arthroplasty was mainly due to DDH. Median follow-up period was 15 years with 22/45 prostheses developing aseptic loosening (event rate 48.9%). Due to a small sample size, certain genotypes were observed in only a few patients: 5 patients (5 prostheses) had T/T genotype at position 29 in the *TGF-β1* signal sequence, 2 subjects (2 prostheses) had G/G genotype at position -308 in the *TNF-α* promoter, 3 subjects (4 prostheses) had G/G and 6 subjects (6 prostheses) had G/C genotype at position -572 in the *IL-6* promoter (Figure 1). Survivorship data for the entire cohort are summarized in the life table (Table 3), and Figure 1 illustrates development of cumulative hazards of aseptic loosening by genotype.

Prosthesis survivorship was analyzed in 4 separate regression models that differed in the number of levels *per* particular independent variable-polymorphic site (Table 4). "Larger" models (Model 1, Model 2) were borderline significant ($0.05< p \leq 0.1$), while "smaller" models (Model 3, Model 4) were statistically significant ($p<0.05$) (Table 4). Hazard ratio for age at THA <46 years (vs. ≥46 years) was consistently around 2-2.5 across all models, and was, within the analyzed sample, not statistically significant (Model 1, Model 2) or was borderline significant ($0.05< p \leq 0.1$) (Model 3, Model 4) (Table 4, Figure 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, Figure 2).

With adjustment for age, sex, reason for THA and other investigated polymorphic sites, the main effect of factor "*TGF-β1* signal sequence ($^{29}T \rightarrow C$)" 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) (Figure 1). When "*TGF-β1* signal sequence (^{29}T→C)" was considered as a 3-level factor (Model 1, Model 3), hazard ratios for genotype T/T (vs. T/C) were around 9 and around 6.5 in the two models, respectively ($p<0.05$) (Figure 2). In Model 2 and Model 4 "*TGF-β1* signal sequence (^{29}T→C)" was considered as a 2-level factor, ie, as T/T (5 subjects, 5 prostheses) vs. "C-allele carriage" (36 subjects, 40 prostheses) (Figure 1). Hazard ratios for genotype T/T were around 8.2 and around 5.7 in the two models, respectively ($p<0.05$) (Figure 2).

The fact that there were only 2 subjects (2 prostheses) with 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.

In Model 1 and Model 2 "*IL-6* promoter (-597G→A)" and "*IL-6* promoter (-572G→C)" were considered as separate 3-level factors, and their main effects were insignificant (Table 4). The most frequent genotypes within the analyzed sample were G/A and C/C at -597 and -572, respectively (Figure 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) (Figure 2). We observed 7/9 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 Model 3 and Model 4, factors "*IL-6* promoter (–597)" and "*IL-6* promoter (–572)" were replaced with a factor "combination

of genotypes *IL-6* (-597) | (-572)" with 2 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). Hazard ratio for the combination G/A -597 | C/C -572 vs. "other" was around 5.4 (Model 3) and around 4.9 (Model 4) ($p<0.05$, respectively) (Figure 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 associated with higher odds of aseptic loosening[10]. This was the first documentation of genetic contribution to this late complication of THA[10]. The genetic component in this condition is likely to include modest contributions by many different polymorphisms, and genes encoding for cytokines involved in 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 (-308 G→A). Unlike association studies that evaluate presence of a certain allele or a genotype and 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]. Since the analyzed group of patients was relatively young, the "cut-off" 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 detection of 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 4

alternative but pre-defined 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 the 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 genotypes ("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: 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 a small sample and the fact that there were only 5 patients (5 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 a 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 relationship between this SNP and 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 (-238 G→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]. A complete linkage disequilibrium between (-597G→A) and (-174G→C) sites[16,25-28], and no disequilibrium[16,25,26,28] or a 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 -597/-572 haplotypes GG (around 50%) and AG (around 40%) as 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 4/41 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 developmental dysplasia of the hip (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 a 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 -597 or -572 genotypes 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 (vs. 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.

In conclusion, the results of this preliminary study point out *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 and suggest that these polymorphic sites deserve to be further investigated in larger studies that would enable conclusions on prevalence of particular genotypes or alleles among THA patients and their clinical relevance.

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

Gen	Primers	N° of cycles / annealing temperature	Fragment length	Polymorphism	Enzymes	Digested fragment length	Ref.
IL-6	Sense primer	35 cycles / at 57°C	169 bp	-597A→C	*Fok* 1	122/47	16
	5'- GCA ACT TTG AGT GTG TCA CG-3'						
	Anti sense primer	35 cycles / at 57°C	163 bp	-572G→C	*Bsr*B 1	101/62	16
	5'-TGA CGT GAT GGA TGC AAC AC-3'						
TGF-ß1	Sense primer 1	40 cycles / at 62°C	346 bp	T^{29} →C			13
	5'-CTC CGG GCT GCG GCT GCT GCT-3'						
	Sense primer 2	40 cycles / at 62°C					
	5'-CTC CGG GCT GCG GCT GCT GCC-3'						
	Anti sense primer	40 cycles / at 62°C					
	5' –GTT GTG GGT TTC CAC CAT TAG-3'						
TNF-α	Sense primer	35 cycles / at 57°C	107 bp	-308G→A	*Nco* 1	42/65	22
	5'-AGG CAA TAG GTT TTG AGG GCC AT-3'						
	Anti sense primer	35 cycles / at 57°C					
	5'-TCC TCC CTG CTC CGA TTC CG-3'						

Table 2. General characteristics of the analyzed patients and hips. Data are counts or median (range).

Number of patients, sex (M/F)	41 (13/28)
Number 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
Body mass index 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, epiphyseolysis)	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

Table 3. Life table for the 45 analyzed endoprotheses. Time since arthroplasty is given as one-year intervals in line with the frequency of the follow-up visits.

Years since surgery	At risk (n)	Failed (n)	Censored (n)	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 to 0.158)
7	44	0	0	0.978	0.022 (0.003 to 0.158)
8	44	1	0	0.978	0.045 (0.011 to 0.180)
9	43	0	0	0.956	0.045 (0.011 to 0.180)
10	43	2	0	0.956	0.091 (0.034 to 0.244)
11	41	3	0	0.911	0.165 (0.078 to 0.345)
12	38	1	0	0.844	0.191 (0.095 to 0.382)
13	37	4	0	0.822	0.299 (0.170 to 0.527)
14	33	3	1	0.733	0.391 (0.235 to 0.651)
15	29	1	5	0.666	0.429 (0.262 to 0.704)
16	23	2	5	0.641	0.527 (0.326 to 0.850)
17	16	1	2	0.578	0.593 (0.368 to 0.957)
18	13	1	8	0.539	0.704 (0.424 to 1.170)
18 +	4	2	2	0.480	1.371 (0.666 to 2.842)

Failed= prostheses presenting with aseptic loosening; censored= “final visit” performed at the particular time since arthroplasty, no signs of aseptic loosening.

Table 4. Summary of proportional hazard regression on time to onset of aseptic instability after total hip arthroplasty in four analyzed models. 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"). Factors age and disease were dichotomized (see Patients and Methods). In Model 1, each factor-polymorphic site had 3 levels (3 possible genotypes). In Model 2, factor *TGF-β1* (29) had 2 levels: "T/T" or "C-allele carriage". In Model 3, factors *IL-6* (–597) and *IL-6* (–572) were replaced by a single factor – 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 Model 2 and Model 3.

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						

Figure 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.

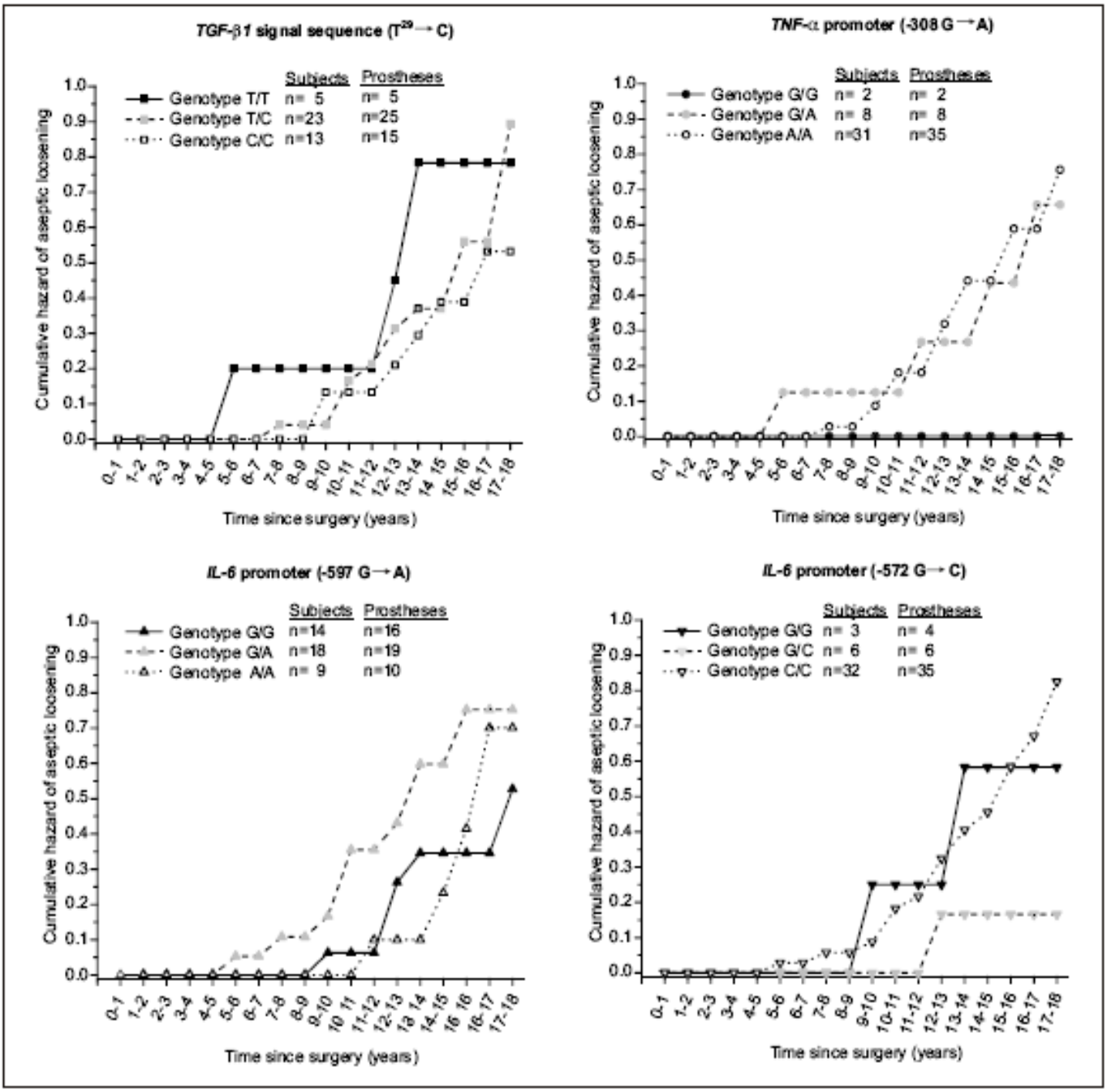
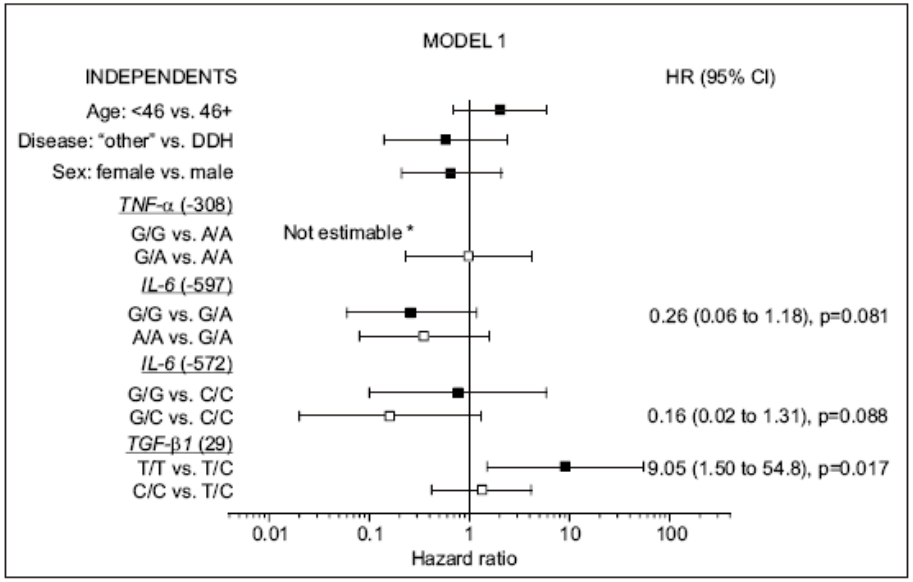
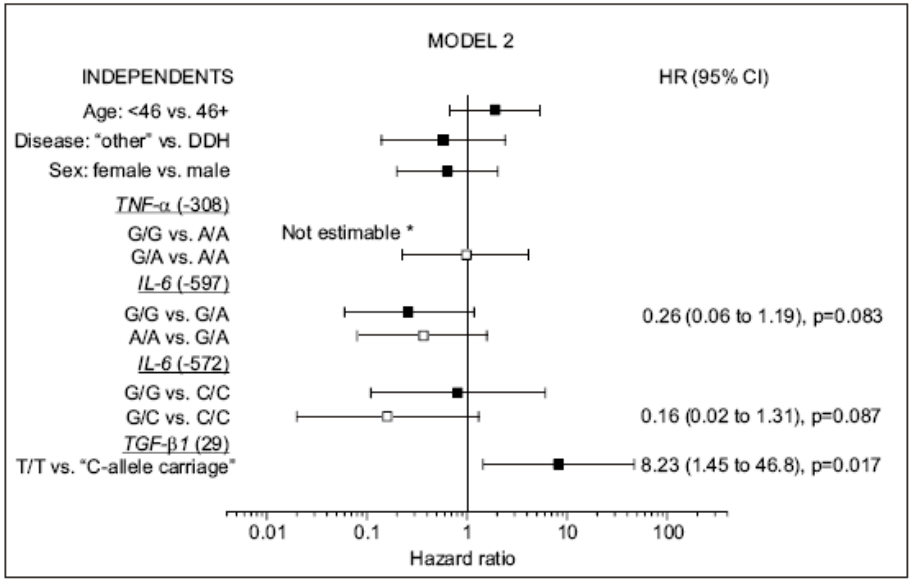
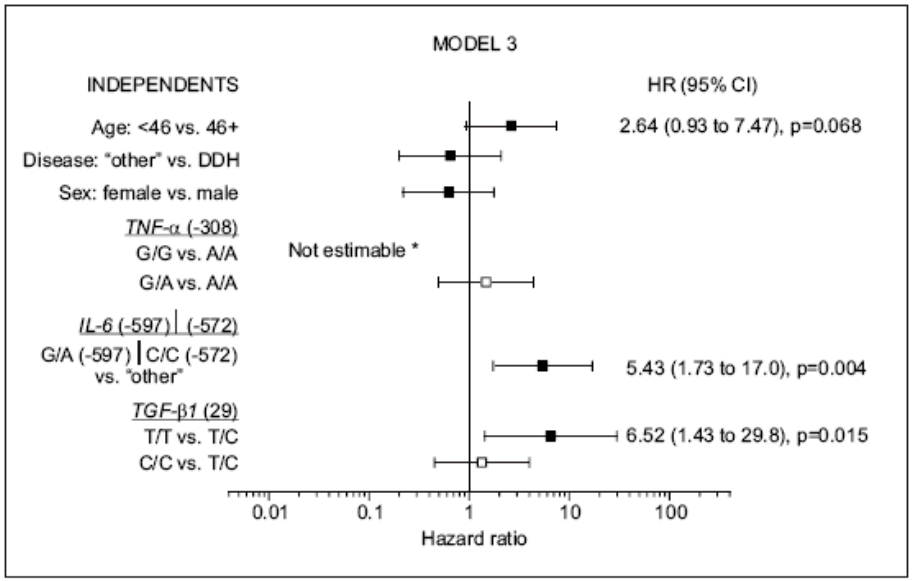


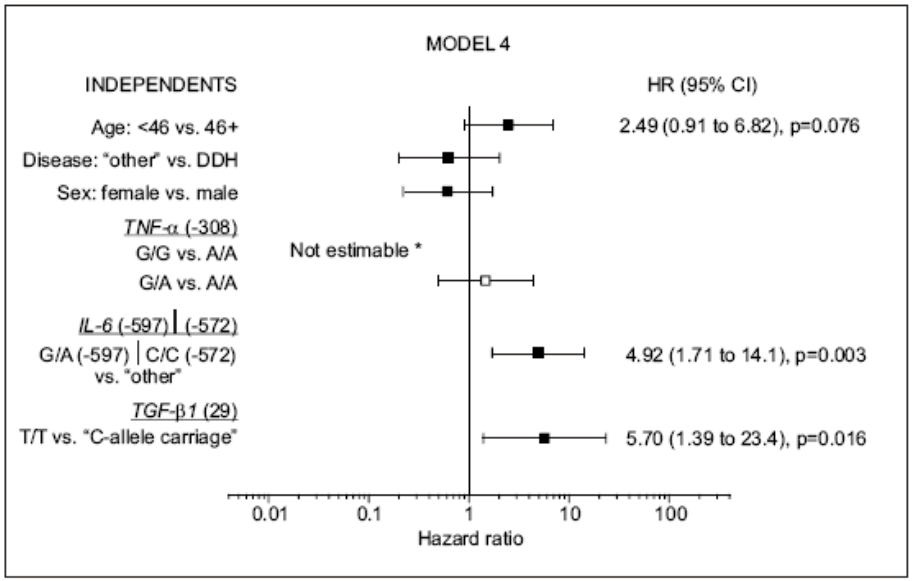
Figure 2. Hazard ratios (HR) from the proportional hazard regression analysis of time to onset of aseptic loosening after total hip arthroplasty in four models depicted in Table 4. For a particular factor-polymorphic site, HR was determined in respect to the most prevalent genotype in the analyzed sample. When factor "*TGF-β1* (^{29}T→C)" had two levels (Model 2, Model 4), HR was determined for genotype T/T in respect to "C-allele carriage". When factor "combination of genotypes *IL-6* (-597) | (-572)" with two levels was included in the analysis (Model 3, Model 4), 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 only for 2 hips, both followed-up for 17 years and not developing aseptic loosening ("censored").


MODEL 1
INDEPENDENTS
HR (95% CI)
Age: <46 vs. 46+
Disease: "other" vs. DDH
Sex: female vs. male
TNF-α (-308)
G/G vs. A/A
Not estimable *
G/A vs. A/A
IL-6 (-597)
G/G vs. G/A
0.26 (0.06 to 1.18), p=0.081
A/A vs. G/A
IL-6 (-572)
G/G vs. C/C
G/C vs. C/C
0.16 (0.02 to 1.31), p=0.088
TGF-β1 (29)
T/T vs. T/C
9.05 (1.50 to 54.8), p=0.017
C/C vs. T/C
0.01
0.1
1
10
100
Hazard ratio


MODEL 2
INDEPENDENTS
HR (95% CI)
Age: <46 vs. 46+
Disease: "other" vs. DDH
Sex: female vs. male
TNF-α (-308)
G/G vs. A/A
Not estimable *
G/A vs. A/A
IL-6 (-597)
G/G vs. G/A
0.26 (0.06 to 1.19), p=0.083
A/A vs. G/A
IL-6 (-572)
G/G vs. C/C
G/C vs. C/C
0.16 (0.02 to 1.31), p=0.087
TGF-β1 (29)
T/T vs. "C-allele carriage"
8.23 (1.45 to 46.8), p=0.017
0.01
0.1
1
10
100
Hazard ratio


MODEL 3
INDEPENDENTS
HR (95% CI)
Age: <46 vs. 46+
2.64 (0.93 to 7.47), p=0.068
Disease: "other" vs. DDH
Sex: female vs. male
TNF-α (-308)
G/G vs. A/A
Not estimable *
G/A vs. A/A
IL-6 (-597) | (-572)
G/A (-597) | C/C (-572) vs. "other"
5.43 (1.73 to 17.0), p=0.004
TGF-β1 (29)
T/T vs. T/C
6.52 (1.43 to 29.8), p=0.015
C/C vs. T/C
0.01
0.1
1
10
100
Hazard ratio


MODEL 4
INDEPENDENTS
HR (95% CI)
Age: <46 vs. 46+
2.49 (0.91 to 6.82), p=0.076
Disease: "other" vs. DDH
Sex: female vs. male
TNF-α (-308)
G/G vs. A/A
Not estimable *
G/A vs. A/A
IL-6 (-597) | (-572)
G/A (-597) | C/C (-572) vs. "other"
4.92 (1.71 to 14.1), p=0.003
TGF-β1 (29)
T/T vs. "C-allele carriage"
5.70 (1.39 to 23.4), p=0.016
0.01
0.1
1
10
100
Hazard ratio