ASSOCIATION OF THE FUNCTIONAL *TGFB1* GENE VARIANTS WITH BONE LOSS AROUND FEMORAL COMPONENT IN TOTAL HIP ARTHROPLASTY

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Background

Periprosthetic osteolysis

Periprosthetic osteolysis (OL) is long-term complication of the total hip arthroplasty (THA) which can result in aseptic loosening and THA reoperation.

Pathogenesis of OL is **complex** – both biological and mechanical factors play an important role.

Wear particles liberated from the prosthetic surfaces **stimulate an inflammatory tissue response** leading to osteolysis.

Substantial **interindividual variability** observed in the severity of OL suggests contribution of **genetic factors.**

Cytokines & Osteolysis

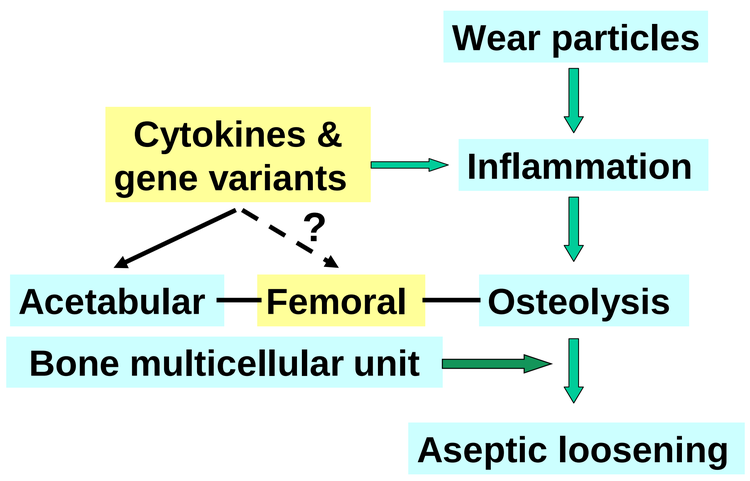
Cytokines are implicated both in inflammatory response and bone resorption pathway.

Production and regulation of cytokines is affected by particular variants of cytokine genes.

We have previously shown that particular **cytokine gene variants are associated with severe acetabular osteolysis** and premature failure of THA[1].

[1]Gallo et al. BMC Med Genet. 2009;10:109

Hypothesis and Objective



Is there any association between the polymorhic variants across a spectrum of genes for cytokines and cytokine receptors with extent of bone loss around the femoral component of THA ?

Methods

1. **Subjects: 205 unrelated Czech patients with cementless type THA** operated on at a single centre stratified according to the severity of femoral bone loss (osteolysis, Saleh´s classification[2])
   Group 1: minor defects (Saleh 1, N=94)
   Group 2: moderate defects (Saleh 2, N=77)
   Group 3: severe defects (Saleh 3-5, N=34)

2. **Genotyping of cytokine / cytokine receptor gene polymorphisms**
   - 22 selected cytokine gene polymorphisms (**Table 1**)
   - Polymerase Chain Reaction with Sequence Specific Primers (PCR-SSP)
   - „The Cytokine Typing Tray kit“, University of Heidelberg

3. **Statistics**
   - conformity of the distribution of genotypes to the Hardy-Weinberg equilibrium
   - differences between allelic, genotype and phenotype („carriage rate“) frequencies: Chi-square test

Table 1
List of investigated cytokine/ cytokine receptor SNPs with their gene location, NCBI reference SNP cluster report (refSNP), and function/location

Cytokine / Receptor	Gene loc.	SNP designation	Ref.SNP	Function/ location	Allele	Allele frequency	
						THA	Czech ref.
IL-1α	2q	-889 T/C	rs1800587	5´UTR	T	0.30	0.30
IL-1β	2q	-511 C/T	rs16944	promoter	T	0.30	0.33
IL-1β	2q	+3962 T/C	rs1143634	coding / synonymous	T	0.24	0.23
IL-1R	2q	pst1 1970 C/T	rs2234650	distal promoter	T	0.33	0.34
IL-1RA	2q	mspa1 11100 T/C	rs315952	coding / synonymous	C	0.34	0.30
IL-4Ra	16p	+1902 G/A	rs1801275	coding / missense	G	0.19	0.20
IL-12	5q	-1188 A/C	rs3212227	3´UTR	C	0.21	0.23
IFNγ	12q	+874 A/T	rs2430561	intron	T	0.47	0.49
TGFβ	19q	Codon 10 T/C	rs1800470	coding / missense	C	0.42	0.47
TGFβ	19q	Codon 25 G/C	rs1800471	coding / missense	C	0.08	0.08
TNF-α	6p	-308 G/A	rs1800629	promoter	A	0.15	0.18
TNF-α	6p	-238 G/A	rs361525	promoter	A	0.04	0.04
IL-2	4q	-330 T/G	rs2069762	promoter	G	0.33	0.31
IL-2	4q	+166 G/T	rs2069763	coding / synonymous	T	0.35	0.35
IL-4	5q	-1098 T/G	rs2243248	promoter	G	0.06	0.06
IL-4	5q	-590 C/T	rs2243250	promoter	T	0.20	0.16
IL-4	5q	-33 C/T	rs2070874	5´UTR	T	0.19	0.17
IL-6	7p	-174 G/C	rs1800795	promoter	C	0.44	0.42
IL-6	7p	nt 565 G/A	rs1800797	promoter	A	0.42	0.42
IL-10	1q	-1082 A/G	rs1800896	promoter	G	0.45	0.47
IL-10	1q	-819 C/T	rs1800871	promoter	T	0.24	0.23
IL-10	1q	-592 C/A	rs1800872	promoter	A	0.24	0.22

Note: The frequency of less common (minor) allele for each SNP is given for patients with THA (N=205) and for the population sample of the Czech healthy subjects (N=150)

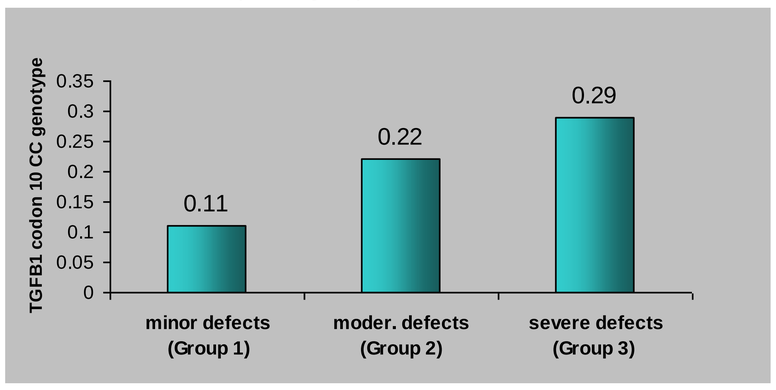
[2]Saleh et al. J Bone Joint Surg Am. 2001;83-A(7):1040-6

Results

Out of investigated cytokine gene variants, the proportion of **transforming growth factor, beta 1** (*TGFB1*) **gene codon 10** (rs1800470) **CC homozygotes increased with the severity of femoral bone defects** (Group 1: 11%, Group 2: 22%, Group 3: 29%, p=0.03; Group 3 *versus* Group 1, p=0.01, OR=3.5, 95% CI: 1.3-9.3; **Figure 1**).

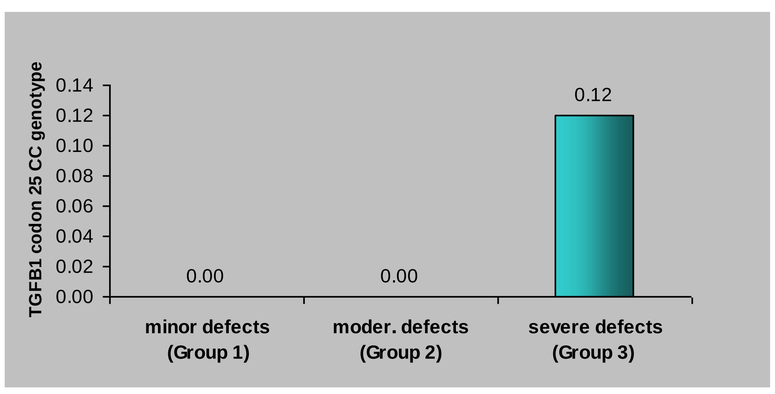
Importantly, the **rare *TGFB1* codon 25** (rs1800471) **CC homozygotes** were observed only in the group of patients with **severe defects** (Group 3, 12%) but not within the groups of patients with moderate or minor defects (p<0.001, **Figure 2**).

Figure 1: Proportion of *TGFB1* codon 10 CC homozygotes in the subgroups of THA patients according to the severity of femoral bone loss (osteolysis)



Overall value for three groups: p = 0.03
Group 3 *versus* Group 1, p=0.01, OR=3.5, 95% CI: 1.3-9.3

Figure 2: Proportion of rare *TGFB1* codon 25 CC homozygotes in the subgroups of THA patients according to the severity of femoral bone loss (osteolysis)



Overall value for comparison among three groups: p < 0.001

Conclusion and Discussion

In conclusion, functional variants of the *TGFB1* gene may confer susceptibility to severe femoral bone defects after THA in a recessive model.

We could speculate that observed association of *TGFB1* variants may be related to the dysregulation of inflammation in response to wear particles and/or insufficiency of bone forming processes around the femoral component of the prosthesis.

Our results may be limited by the small numbers of*TGB1* uncommon homozygotes (namely *TGFB1* codon 25 CC); replication of this study in independent THA cohorts is, therefore, warranted. Further data on the role of TGFbeta and its gene variants in the pathogenesis of osteolysis are desirable as well.

Grant support: MSM 6198959205, IGA MHCR NR9490-3, NS/10260-3 and IGA UP project no. LF_2010_008, CZECH REPUBLIC