Osteoprotegerin gene polymorphism is not associated with prosthetic joint infection after total joint arthroplasty in the Czech population

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Background and aims. Osteoprotegerin (*OPG*; official gene symbol: *TNFRSF11B*) is considered a negative regulator of bone resorption via inhibition of osteoclast differentiation. Further, *OPG* expression has been detected in Prosthetic Joint Infection (PJI) a serious complication limiting the overall outcome of total joint arthroplasty (TJA). As *OPG* may be a candidate molecule for PJI pathogenesis, we investigated whether genetic variation in the *OPG* promoter, namely the SNP at position -163 was associated with PJI.

Methods. *OPG* -163 T/C SNP (rs3102735) was genotyped by polymerase chain reaction with sequence specific primers (PCR-SSP) in 98 Czech patients with PJI and two Czech control groups: 1) aseptic TJA control [251 patients with TJA who did not develop PJI at least 6 yrs. after the surgery] and 2) population control (185 healthy control subjects without TJA).

Results. The distribution of *OPG* -163 SNP genotypes complied with the Hardy-Weinberg equilibrium in all three groups. The allele frequencies of *OPG* -163 SNP were similar in patients with PJI (minor allele frequency: 0.14), those with aseptic TJA (0.13) and population controls (0.14, $P > 0.05$). Further, there was no significant difference in genotype or phenotype frequency (carriage rate) between patients with PJI and both control groups ($P > 0.05$).

Conclusions. In a Czech population, the *OPG* -163 T/C SNP has not been found to be associated with PJI.

Key words: osteoprotegerin, RANKL-RANK-*OPG* axis, prosthetic joint infection, DNA sequence variant, *TNFRSF11B*, arthroplasty, single nucleotide polymorphism, osteoimmunology

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INTRODUCTION

Prosthetic joint infection (PJI) is a serious complication threatening both the early and late period after total joint arthroplasty (TJA). Current estimates suggest that despite careful management to avoid all sources of infection, PJI can develop in up to 1.7% of primary hip arthroplasties and 2.5% of primary knee arthroplasties¹. Given that each year at least two millions TJAs are performed worldwide, the number of infected cases is enormous and is predicted to increase².

PJI is caused by bacterial colonisation of the prosthetic joint. Regardless of particular route of infection it is clear that host factors may increase the risk of PJI by compromising the immune response³. Bacteria and their products are also potent inducers of bone resorption. Experimentally, significant reduction in bone mineral density shortly after the beginning of *Staphylococcus aureus* infection of the bone has been revealed⁴. This was effectively prevented by inhibition of RANKL (receptor activator of nuclear factor – kappa B ligand) signalling. This and other experiments support the clinical observation of a tight association between the development of infection and increased resorption of periprosthetic bone⁵. For this reason, cytokine ligands and the corresponding

receptors that regulate osteoclast accumulation, maturation and viability, are probably involved in the complex pathogenesis of PJI. The axis RANK-RANKL-*OPG* is the key regulator of the bone multicellular unit, and genetic factors related to this axis have been shown to affect bone mineral density⁶⁻⁸, osteoporosis⁷ and periprosthetic osteolysis⁹. A recent study demonstrated deregulation of *OPG* expression in osteoblasts after *Staphylococcus aureus* infection¹⁰. Also, expression of *OPG* was observed in periprosthetic interface membranes retrieved from septic prostheses¹¹.

Osteoprotegerin (*OPG*; official gene symbol: *TNFRSF11B*) acts as a soluble decoy receptor that blocks binding of RANKL to its receptor (RANK) inhibiting osteoclast differentiation. The human *OPG* represents a single copy gene located on chromosome 8 (8q24). It spans 29 kb and consists of 5 exons, 4 introns, and is transcribed into four transcripts, 2.4 kb one representing the major transcript¹². There is a degree of genetic variation in the gene with at least 10 SNP localised mostly within the 5' flanking region and also exons/introns¹³. A plausible contribution of a genetic variation in the *OPG* gene to PJI has so far been investigated only in a single centre and with inconsistent results^{9,14}. In agreement with the current rules for conducting genetic association stud-

ies¹⁵, and especially those relevant to areas of rheumatology and osteoimmunology¹⁶, we therefore investigated if *OPG*-163 T/C polymorphism is associated with PJI in a Czech population.

MATERIAL AND METHODS

Study population

OPG-163 SNP T/C (rs3102735) was genotyped in 98 patients with PJI and two control groups: i) aseptic TJA control (251 patients with TJA who did not develop PJI at least 6 yrs. after the surgery) and ii) population control (185 healthy control subjects without TJA). Of 98 infected cases, 16 patients were recruited at the Dept. of Orthopaedics Frýdek Místek and Znojmo, Czech Republic. Both patients and controls were unrelated individuals of Czech origin. Clinical and laboratory characteristics of patients with PJI and controls are listed in Table 1. PJI was diagnosed according to the following criteria¹⁷: i) presence of sinus tract communicating with a joint and/or intra-articular pus; ii) coincidentally positive results of histological examination (five or more neutrophils per high power field) and culture of intraoperative samples; iii) if only intraoperative culture or histological results were positive, then at least two of the following signs had to be present: high clinical suspicion of infection (acute onset, fever, erythema, edema, persistent local pain, early prosthetic failure, wound healing disturbances, etc.), erythrocyte sedimentation rate >30 mm/hr, C-reactive protein elevated more than 1.5 times above the laboratory reference value, positive 99m Technetium leukocyte scintigraphy (the last criterion included only in patients from the University Hospital Olomouc). Patients without PJI did not fulfil these criteria.

Informed consent for the anonymous use of their DNA for the purposes of this study was obtained from all subjects. The study was performed with the approval of the local Ethics Committee.

Genotyping

Polymerase chain reaction with sequence specific primers (PCR-SSP) was used to genotype *OPG*-163 T/C SNP (rs3102735). Standard and mutant alleles were amplified in two separate reactions. The constant reverse primer (5' tcaccttccttccttgaatc 3') was used for amplification with forward specific primers either for the standard allele (5' gttegtgtctccccatt 3') or for the minor allele (5'gttegtgtctccccattc 3'). Reaction conditions and internal controls for PCR-SSP were adopted from Phototyping methodology¹⁸, the cycling profile consisted of initial denaturation (96 °C, 1 min) followed by first five cycles (96 °C - 20 s, 70 °C - 45 s, 72 °C - 25 s), further 21 cycles (96 °C - 25 s, 65 °C - 50 s, 72 °C - 30 s) and final four cycles (96 °C - 30 s, 55 °C - 1 min, 72 °C - 1 min 30 s).

Statistics

The significance of differences in allelic, genotype and phenotype frequencies between all groups was determined

by means of the χ^2 test applying the Woolf-Haldane correction for small numbers. The relative ratio (RR), 95% confidence interval (CI) and *P*-value were calculated. The statistical power was determined according to the protocol described elsewhere¹⁹.

RESULTS

The distribution of alleles, genotypes, and phenotypes of *OPG*-163 SNP in patients with PJI and two control samples is presented in Table 2. The distribution of *OPG*-163 SNP genotypes complied with the Hardy-Weinberg equilibrium in all three groups. The allele frequencies of *OPG*-163 SNP were similar in the patients with PJI (minor C allele frequency: 0.14), those with aseptic TJA (0.13) and population controls as well (0.14, *P*>0.05). Further, the genotype and phenotype frequency (carriage rate) did not differ between the patients with PJI and either of the two control groups (*P*>0.05).

When the patients with PJI were stratified according to the affected joint, there was no association between *OPG*-163 SNP and PJI of Total Hip Arthroplasty - C allele frequency was equal in both aseptic THA and in THA/PJI groups: 0.12; *P*>0.05). Similarly, the distribution of *OPG*-163 SNP did not differ between the patients with aseptic Total Knee Arthroplasty (TKA, C allele frequency: 0.14) and those with TKA/PJI (0.16; *P*>0.05).

The statistical power of the present study to replicate observations reported for the British population in the initial report⁹ was higher than 99%. The power to detect differences between cases and controls was 94% if aiming at an odds ratio (OR) 2.0, and 82% for OR 1.75.

DISCUSSION

The results show no association between -163 T/C single nucleotide polymorphism in the Osteoprotegerin gene and the risk of prosthetic joint infection. This finding may be interpreted in two ways, either 1) "individually" as an independent study of a candidate SNP in the *OPG* gene in the Czech population, or 2) in the context of previously reported controversial data from the British population: Here, Malik et al.⁹ first reported *OPG*-163 as a risk factor for PJI. However only after three years Malik et al.¹⁴ published a brief note that this was/might not be true due to "inadvertent data manipulation".

Given the fact, that the latter brief note¹⁴ was not formulated as a typical erratum statement and thus has not been linked to the original report⁹ in databases such as PubMed, the association between the investigated polymorphism and PJI can still be considered to be present in the "universe" of genetic association data as it may be exemplified e.g. by the current review²⁰. Thus, in our opinion, and in accordance to the appropriate "guidelines" (ref.^{15-16,20}) the replication of these data coming from a single centre is required to prove there is adequate statistical power. If we understood our current work as a

Table 1. Characteristics of Czech patients with PJI and two control groups of the patients with aseptic TJA and Czech population controls.

	Population controls n=185	Aseptic TJA n=251	PJI n=98
Gender; men/women	87/98	78/173	49/49
Age at index surgery; years	28 (24-34)	49 (43-54)	67 (58-73)
Joint; hip/knee/other*	0	203/48/0	43/52/3
BMI (median)	n.a.	28 (25-30)	29 (26-30)
Primary diagnosis:			
Osteoarthritis	0	91	74
Dysplastic joint	0	113	10
Inflammatory joint	0	13	3
Other diagnosis	0	34	11
Comorbidity:			
Nothing	185	157	56
Diabetes mellitus	0	22	12
Other+	0	72	30
Intraoperative culture:			
Staphylococci	n.a.	0	58
Other pathogens	n.a.	0	19
Negative	n.a.	251	21
Type of PJI:			
Early	n.a.	n.a.	24
Delayed	n.a.	n.a.	43
Haematogenous	n.a.	n.a.	22
Other	n.a.	n.a.	9

Legend: Continuous data presented as median with interquartile (1st to 3rd) range in parentheses. * Shoulder, elbow, + e.g. rheumatoid arthritis and nicotine addiction, n.a., not applicable; BMI, body mass index.

Table 2. Genotype, allele and phenotype (carriage rates) frequencies of the *OPG* -163 SNP in the patients with PJI and two control groups of the patients with aseptic TJA and Czech population controls.

Frequency	Genotype			Allele		Phenotype	
-163 T/C rs3102735	TT	TC	CC	T	C	T	C
Population controls (n=185)	0.72(133)	0.28(51)	0.00(1)	0.86(317)	0.14(53)	0.99(184)	0.28(52)
Aseptic TJA (n=251)	0.76(191)	0.23(57)	0.01(3)	0.87(439)	0.13(63)	0.99(248)	0.24(60)
PJI (n=98)	0.73(72)	0.25(24)	0.02(2)	0.86(168)	0.14(28)	0.98(96)	0.27(26)

The data are given as frequency of a particular genotype/allele/phenotype with absolute numbers in parentheses.

replication, it was important to note that we investigated a larger number of patients with PJI (n=98) than the original British study (n=63). Thus the statistical power of our study was more than adequate (>99%) to replicate the association of PJI with *OPG* -163 SNP observed in the original, first British report⁹.

It may be argued that despite the low public visibility of the “corrected” data, a replication study cannot be performed in the present. However, if we consider our study merely as an individual undertaking aimed at the

assessment of the plausible association between *OPG* -163 T/C SNP and PJI, it has very good power (94%) to detect association in the range of OR values around 2.0, and is still adequately powered around OR 1.7 (81%); the acceptable limit being 80% (ref.¹⁹). For the detection of weaker associations (OR <1.7), however, the number of study subjects will have to be increased which we acknowledge is a limitation of the study.

We cannot exclude the possibility that the observed lack of genetic association between *OPG* -163 SNP and

PJI in our Czech cohort was due to inappropriately targeted selection of candidate SNP. Indeed, it has been very recently reported that the *OPG* -163 T/C may not affect the expression of the *OPG* gene⁶, however this was an isolated *in vitro* report. In this context, there are more SNPs located on the *OPG* gene¹³. However the data on their functionality and mutual relationships are mostly limited to non-Caucasian populations^{21,22}.

CONCLUSION

In conclusion, even if the present study had sufficient statistical power both as an individual “exercise” and/or a replication study, it provided no further evidence that *OPG* -163 T/C SNP is associated with prosthetic joint infection. After more information is obtained on the spectrum of genetic variation in the *OPG* gene, analyses with further SNPs may allow us to draw definite conclusion about a possible role of Osteoprotegerin genetic variation in PJI.

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CONFLICT OF INTEREST STATEMENT

The authors state that there are no conflicts of interest regarding the publication of this article.

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