Genetic variation in key molecules of the Th-17 immune response is not associated with risk for prosthetic joint infection in a Czech population

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Background and aims. Prosthetic Joint Infection (PJI) is a serious complication of Total Joint Arthroplasty (TJA). The Th-17 immune response characterised by IL (interleukin)-17A, IL-17F, IL-23, chemotactic cytokines and their receptors, plays a prominent role in the immune response to invading bacteria. In addition, high expression of IL-17A has been reported in PJI. The aim of this study was to investigate whether genetic variation in the key molecules of the Th-17 immune response can affect the risk for PJI.

Methods. Altogether ten Single Nucleotide Polymorphisms (SNPs) of *IL17A* (rs2275913), *IL17F* (rs763780), *IL4* (rs2243250), *IL12A* (rs583911), *IL12B* (rs3212227 and (rs17860508)), *IL23R* (rs7517847), *CXCL1* (rs4074), *CXCL5* (rs425535) and *CXCR2* (rs2230054) genes were genotyped by PCR with sequence specific primers (SSP) in 98 patients with PJI and two control groups 1) an aseptic TJA control (253 patients with TJA that did not develop PJI at least 6 yrs. after the surgery) and 2) a population control (185 healthy control subjects without TJA).

Results. Allele, genotype and phenotype frequencies of investigated SNPs did not differ between the patients with PJI and control patients with aseptic TJA ($P>0.05$). There was no difference in the distribution of tested SNPs between patients with PJI and population controls without TJA ($P>0.05$) or between the two controls groups ($P>0.05$).

Conclusions. We cannot nominate any of studied polymorphisms in *IL17A*, *IL17F*, *IL4*, *IL12A*, *IL12B*, *IL23R*, *CXCL1*, *CXCL5* and *CXCR2* genes as risk factors for PJI in the Czech TJA patients examined.

Key words: prosthetic joint infection, total joint arthroplasty, infection, sepsis, interleukin-17, chemokines, chemokine receptors, single nucleotide polymorphisms

Received: December 14, 2011; Accepted: February 15, 2012; Available online: April 18, 2012

<http://dx.doi.org/10.5507/bp.2012.023>

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INTRODUCTION

Prosthetic Joint Infection (PJI) is a serious complication after Total Joint Arthroplasty (TJA) that usually leads to clinical failure requiring surgical revision and long-term antibiotic therapy. The cause of PJI is the presence of microorganisms such as coagulase-negative *staphylococci* and *Staphylococcus aureus* (ref.¹). However, exposure of implant and periprosthetic environment to low-dose bacteria is not adequate cause of PJI: not all TJA patients and not everyone with confirmed *Staphylococcus aureus* bacteraemia develop PJI (ref.^{2,3}). We, in line with our previous research in the field of periprosthetic osteolysis⁴ and other investigators⁵, hypothesise that PJI could be a result of the harmful combination of the bacterial exposure and complex individual susceptibility to PJI in which genetic variants associated with a weakened immune response may play an important role.

The cytokines and chemokines related to the Th-17 immune response are candidate genes for increased susceptibility to PJI as they play a prominent role in the immune protection against microorganisms⁶⁻⁸. Further, IL(interleukin)-17 and the chemokines associated with

the Th-17 immune response are up-regulated in patients with PJI suggesting the involvement of the Th-17 immune pathway in host response in PJI (ref.⁹). Expression and function of the cytokines and chemokines related to Th-17 immune response are known to be affected by genetic factors such as Single Nucleotide Polymorphisms (SNPs) (ref.¹⁰). However, to date there are no data on the SNPs within the key molecules of Th-17 immune response in patients with PJI. We therefore investigated whether selected polymorphisms of the genes associated with Th-17 signalling, namely *IL17A*, *IL17F*, *IL4*, *IL12A*, *IL12B*, *IL23R*, *CXCL1*, *CXCL5* and *CXCR2*, could be associated with the risk of PJI.

MATERIALS AND METHODS

Study population

In three Czech collaborating orthopaedic centres (Frýdek Místek, Znojmo and Olomouc) we recruited 98 patients with PJI (Table 1) and two control groups (only from the Olomouc centre): i) 253 patients with aseptic TJA that did not develop PJI at least 6 yrs. after

the surgery and ii) 185 population controls without TJA (Olomouc). PJI was diagnosed according to the following criteria¹¹: 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.

All patients and controls were unrelated individuals of Czech origin. 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

The investigated polymorphisms were genotyped by the polymerase chain reaction with sequence specific primers (PCR-SSP). Nucleotide sequences of primers are shown in Table 2. Reaction conditions and internal controls were adopted from the Phototyping methodology¹², and the protocol is described elsewhere¹³.

Statistics

We used χ^2 -analysis to test for a deviation of the genotype distribution from Hardy-Weinberg equilibrium. The significance of differences in allelic, genotype and phenotype frequencies between all groups was determined by means of the χ^2 test applying Woolf-Haldane correction for small numbers. The statistical power of the present study was determined according to the protocol described elsewhere¹⁴.

RESULTS AND DISCUSSION

Allele, genotype and phenotype frequencies of ten investigated polymorphisms located within nine genes encoding for IL-17A, IL-17F, IL-4, IL-12A, IL-12B, IL-23R, CXCL1, CXCL5 and CXCR2 molecules did not differ between the patients with PJI and control patients with aseptic TJA ($P>0.05$ for all SNPs; Table 3). To strengthen our data, we recruited second control group (Czech population controls) and there was no difference in the distribution of 10 polymorphisms between the patients with PJI and the population controls without TJA. Moreover, the frequencies of the investigated polymorphisms within the *IL17A*, *IL17F*, *IL4*, *IL12A*, *IL12B*, *IL23R*, *CXCL1*, *CXCL5* and *CXCR2* genes corresponded to their distribution in other Caucasian populations¹⁵⁻²¹.

Table 1. Characteristics of healthy controls, the patients with aseptic TJA and with PJI.

	Population controls n=185	Aseptic TJA n=253	PJI n=98
Gender; men/women	87/98	79/174	49/49
Age; years*	28 (24-34)	49 (43-51)	67 (58-73)
Joint; hip/knee/other [#]	0	205/48/0	43/52/3
BMI (median)	n.a.	28 (25-30)	29 (26-30)
Primary diagnosis:			
Osteoarthritis	0	91	74
Dysplastic osteoarthritis	0	114	10
Inflammatory osteoarthritis	0	13	3
Traumatic and other diagnosis	0	35	11
Intraoperative culture:			
Staphylococci	n.a.	0	58
Other pathogens	n.a.	0	19
Negative	n.a.	253	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.

Genetic variation in the key molecules of the Th-17 immune response was investigated here because the Th-17 pathway contributes significantly to antibacterial host response employing several mechanisms. One is IL-17A-induced release of CXCL1 and CXCL5 chemokines that stimulates neutrophil chemotaxis via a common CXCR2 receptor²². As a result, neutrophils accumulate prolifically in periprosthetic tissues and this is considered strong evidence for PJI (histopathological diagnostics of PJI). Additionally the up-regulation of IL-17A and CXCL5 has been found in the synovial fluid of patients with PJI (ref.⁹). Other molecules are known to regulate the development (IL-4, IL-12A subunit of IL-35) or maintenance (IL-23 receptor and 12B subunit of its ligand IL-23) of the Th-17 immune response^{8,23,24}.

We recruited a large number of well-defined patients with PJI in order to assess the role of genetic variation in the key molecules of the Th-17 immune response in the pathogenesis of PJI. Despite sufficiently high statistical power (see Table 2), none of the polymorphisms within *IL17A*, *IL17F*, *IL4*, *IL12A*, *IL12B*, *IL23R*, *CXCL1*, *CXCL5* and *CXCR2* genes affected susceptibility to PJI in our patients after TJA. In the case of *IL17F* polymorphism, however, we could have missed the genetic association with PJI because this polymorphism is relatively rare in Caucasian population¹⁵.

The negative data on the association of both *IL12B* polymorphisms and *CXCR2* polymorphism with PJI

should be interpreted carefully as these polymorphisms did not comply with the Hardy-Weinberg equilibrium (HWE). Two *IL12B* SNPs did not follow the HWE in the control group of patients with aseptic TJA, but both were in line with HWE among population controls (Table 3). Regarding the *CXCR2* gene, the genotype distribution of *CXCR2* SNP did not follow HWE in the patients with PJI (Table 3). In both *IL12B* polymorphisms and *CXCR2* SNP, the deviation from HWE could occur by chance (random fluctuation) when we tested the distribution of 10 SNPs in 3 groups (30 tests in total) (ref.²⁵).

The variability in the phenotype of PJI patients should be further discussed in line with severe sources of heterogeneity between cases and controls which is a common limitation of genetic association studies leading eventually to false negative results^{26,27}. First, unavoidable heterogeneity at least in comorbidities and microbial cause of infection in our PJI patients could play a role (Table 1). These comorbidities have their own genetic component²⁸ that may contribute to PJI pathogenesis. Second, the type of affected joint could influence the risk of PJI. Here, none of the investigated polymorphisms affected the risk of either hip or knee PJI separately (data not shown). Finally, as in other genetic sub-analyses of case-control studies the small sample of PJI patients in individual subgroups can distort the results.

Table 2. Nucleotide sequence of the primers used for PCR-SSP genotyping of ten investigated SNPs.

SNP §statistical power	Sequence of primes	SNP §statistical power	Sequence of primers
IL-17A rs2275913 99%	gaggtcatagaagaatctctc gaggtcatagaagaatctctt acctacatgttacttcaaactt	IL-12B rs17860508 99%	ctgtctccgagagagggc gtctccgagagggctctaa ctgccctcgggactgac
IL-17F rs763780 50%	tcaccctgtcatccacca tcaccctgtcatccaccg agtacatacacatacattgtg	IL-23R rs7517847 99%	tcacatttccaaggccg tcacatttccaaggcct tgtggcaaatgccctgaac
IL-4 rs2243250 97%	ctaccacgactggggg ctaccacgactgggga cctgatcacaacattgcatttc	CXCL1 rs4074 99%	tcctccagcctctagagttc tcctccagcctctagagttt ggacaaacagcaacaggttct
IL-12A rs583911 99%	ggaactgttaaggtcacagtt ggaactgttaaggtcacagtc agtgccctgccaccattag	CXCL5 rs425535 88%	atcattttgggatgaactccc atcattttgggatgaactcct cagcggtagagcgcgatg
IL-12B rs3212227 98%	tttcaatgagcatttagcatct tttcaatgagcatttagcatcg agacattgctgttacaatgt	CXCR2 rs2230054 99%	gttgtagggcagccagcag gttgtagggcagccagcaa cgtctactcatccaatgttag

Legend: § Statistical power to detect OR=2.00 at alpha=5%. Allele frequencies of tested SNPs in control group of the patients with aseptic TJA were used as reference data for the calculation of statistical power.

Table 3. Distribution of 10 investigated SNPs in PJI patients and two control groups.

Gene	Population controls			Aseptic TJA			PJI		
SNP	Genotype f. (%)	Allele f. (%)	Phenotype f. (%)	Genotype f. (%)	Allele f. (%)	Phenotype f. (%)	Genotype f. (%)	Allele f. (%)	Phenotype f. (%)
<i>IL17A</i> rs2275913 G/A	GG 37 GA 51 AA 12	G 63 A 37	G 88 A 63	GG 38 GA 46 AA 16	G 61 A 39	G 84 A 62	GG 39 GA 45 AA 15	G 62 A 38	G 85 A 61
<i>IL17F</i> rs763780 T/C	TT 88 TC 12 CC 0	T 94 C 6	T 100 C 12	TT 94 TC 6 CC 0	T 97 C 3	T 100 C 6	TT 93 TC 7 CC 0	T 96 C 4	T 100 C 7
<i>IL4</i> rs22432597 C/T	CC 70 CT 26 TT 4	C 83 T 17	C 96 T 30	CC 63 CT 34 TT 3	C 80 T 20	C 97 T 37	CC 66 CT 31 TT 3	C 82 T 18	C 97 T 34
<i>IL12A</i> rs583911 A/G	AA 30 AG 54 GG 16	A 57 G 43	A 84 G 70	AA 33 AG 53 GG 14	A 60 G 40	A 86 G 67	AA 35 AG 55 GG 10	A 62 G 38	A 90 G 65
<i>IL12B*</i> rs3212227 A/C	AA 63 AC 31 CC 6	A 79 C 21	A 94 C 37	AA 63 AC 30 CC 7	A 78 C 22	A 93 C 37	AA 66 AC 33 CC 1	A 83 C 17	A 99 C 34
<i>IL12B*</i> rs17860508 GC/ CTCTAA	11 28 12 48 22 24	1 52 2 48	1 76 2 72	11 33 12 43 22 24	1 55 2 45	1 76 2 67	11 32 12 53 22 15	1 58 2 42	1 85 2 68
<i>IL23R</i> rs7517847 T/G	TT 43 TG 42 GG 15	T 64 G 36	T 85 G 57	TT 36 TG 48 GG 16	T 60 G 40	T 84 G 64	TT 35 TG 44 GG 21	T 57 G 43	T 79 G 65
<i>CXCL1</i> rs4074 G/A	GG 43 GA 45 AA 12	G 66 A 34	G 88 A 57	GG 43 GA 46 AA 11	G 66 A 34	G 89 A 57	GG 36 GA 55 AA 9	G 63 A 37	G 91 A 64
<i>CXCL5</i> rs425535 G/A	GG 81 GA 19 AA 0	G 91 A 9	G 100 A 19	GG 81 GA 19 AA 0	G 90 A 10	G 99 A 19	GG 77 GA 23 AA 0	G 89 A 11	G 100 A 23
<i>CXCR2*</i> rs2230054 C/T	CC 25 CT 48 TT 27	C 49 T 51	C 73 T 75	CC 28 CT 44 TT 30	C 50 T 50	C 72 T 72	CC 33 CT 37 TT 31	C 51 T 49	C 69 T 67

Legend: *rs3212227, aseptic TJA, $P_{HWE}=0.03$; rs17860508, aseptic TJA, $P_{HWE}=0.04$; rs2230054, PJI patients, $P_{HWE}=0.01$

CONCLUSION

None of the examined polymorphisms in *IL17A*, *IL17F*, *IL4*, *IL12A*, *IL12B*, *IL23R*, *CXCL1*, *CXCL5* and *CXCR2* genes were shown to be risk factors for PJI in Czech patients. If there is a real genetic background to increased susceptibility for PJI, the negative data reported here will help other genetic studies to target real genetic factors associated with PJI. The dissemination of such negative genetic association data is therefore valuable and their publication is in line with the policy of genetic research^{29,30}.

ACKNOWLEDGEMENTS

Technical help of the staff of Department of Orthopaedics, University Hospital Olomouc, is gratefully acknowledged. The authors also express their gratitude to the medical staff of the Orthopaedics clinics in Znojmo and Frýdek Místek providing patients' samples for a part of this study. The investigations were supported by Czech governmental funding GACR 310/09/P377, and in part by IGA MHCR NS/10260.

CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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