Influence of Interleukin 1α (IL-1α), IL-4, and IL-6 Polymorphisms on Genetic Susceptibility to Chronic Osteomyelitis

Aspasia Tsezou,1,2,3* Lazaros Poultsides,4 Fotini Kostopoulou,1 Elias Zintzaras,5 Maria Satra,3 Sofia Kitsiou-Tzeli,6 and Konstantinos N. Malizos2,4

University of Thessalia, Medical School, Laboratory of Cytogenetics and Molecular Genetics, Larissa, Greece;1 Institute of Biomedical Research and Technology, Larissa, Greece;2 University of Thessalia, Medical School, Department of Biology, Larissa, Greece;3 University of Thessalia, Medical School, Department of Orthopaedics, Larissa, Greece;4 University of Thessalia, Medical School, Department of Bioinformatics, Larissa, Greece;5 and University of Athens, Department of Medical Genetics, Athens, Greece6

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The association between cytokine gene polymorphisms and chronic osteomyelitis was investigated in order to determine whether genetic variability in cytokine genes predisposes to osteomyelitis susceptibility. Significant genotypic and allelic associations were observed between interleukin 1α (IL-1α) −889-C/T, IL-4 −1098-G/T and −590-C/T, and IL-6 −174-G/C polymorphisms and osteomyelitis in the Greek population, pointing towards their potential involvement in osteomyelitis pathogenesis.

Osteomyelitis (OM) is a bone infection characterized by progressive inflammatory destruction of the bone, bone necrosis, and induction of new bone apposition at the site of infection (11, 16).

Increased levels of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor alpha released by the host have been reported in OM patients (4, 7). Specifically, cytokines such as IL-1β, IL-6, tumor necrosis factor alpha and IL-4 are directly involved in the bone desorption and osteoclast activity regulation that occur in OM and may therefore play a role in its pathogenesis (1–3, 10, 13, 14).

Single nucleotide polymorphisms (SNPs) are important causes of genetic variation, and have been associated with susceptibility to infectious diseases (8). The association between cytokine gene polymorphisms and chronic OM was investigated in order to determine whether genetic variability in cytokine genes could predispose to OM susceptibility.

A total of 191 Greek individuals participated in this case-control study. The OM group contained 81 patients, consisting of 42 men (ages 47.3 ± 9.1 years; range, 19 to 77 years) and 39 women (ages 58.6 ± 8.2 years; range, 22 to 85 years) hospitalized between February 2005 and March 2007 at the Orthopaedic Department of the University Hospital of Larissa, Greece. The control population contained 110 healthy subjects, consisting of 62 men (ages 56 ± 8.4 years; range, 30 to 79 years) and 48 women (ages 62 ± 9.3 years; range, 35 to 80 years), who had no history of musculoskeletal infection. The OM and control groups were age and sex matched.

OM was diagnosed by clinical, roentgen graphic, computerized tomography, magnetic resonance imaging, and isotopic bone imaging criteria. Surgical and sinus tract pus samples were cultured.

Microorganisms isolated from OM patients are shown in Table 1. Fifty-two of the patients had one or more comorbidities, such as diabetes mellitus, cancer, autoimmune diseases, or renal failure. The number of admissions (≥3), number of inpatient days (≥20), number of surgical operations performed (≥3), and the type of microorganism were also evaluated and correlated with genetic polymorphisms.

Each participant gave informed consent, which was approved by the Ethics Committee of the University of Thessalia.

Genomic DNA was extracted from peripheral blood using a commercially available kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Eighteen cytokine polymorphisms were determined in a PCR with a sequence-specific primer (PCR SSP) assay (Protrons HLA cytokines 2).

The association between genotype distribution and disease was tested using a chi-square test. The associations were expressed as unadjusted and adjusted odds ratios (ORs) and their corresponding 95% confidence intervals (CI) using logistic regression. Controls were tested for Hardy-Weinberg equilibrium using an exact test (17). Data analysis was performed using GDA V1.0 (http://lewis.eeb.uconn.edu/lewishome/software.html) and GLIM3.77 (Royal Statistical Society, United Kingdom).

The minimum detectable ORs under the log additive model with a power of ≥95% and a significance level of 5% were calculated for the T allele of IL-1α (−889 C/T) and IL-4 (−1098 G/T and −590 C/T) and the C allele of IL-6 (−174 G/C) using Quanto 1.1.5 (Table 2).

Significant associations were observed only between IL-1α −899-C/T, IL-4 −1098-G/T and −590-C/T, and IL-6 −174-G/C polymorphisms and OM (P < 0.01) (Table 3). The controls were in Hardy-Weinberg equilibrium for the polymorphisms studied (P > 0.05). Individuals with TT at the IL-1α −889-C/T polymorphism had almost sevenfold increased risk for OM development, with this association remaining significant even after adjustment for age and sex (Table 4). Our finding is in agreement with a previous study, reporting that IL-1α −889-C/T polymorphism is a risk factor for OM and that it is associated with a younger age at diagnosis of OM (2). IL-1 has a profound effect on bone physiology, especially osteoclastogenesis, which creates giant cells that are able to resorb (9).
As bone loss at the site of infection is a characteristic of OM, it could be suggested that this cytokine is associated with OM development.

We also found that two polymorphisms in the IL-4 gene (–1098 G/T and –590 C/T) were significantly associated with increased risk of OM and that individuals carrying the T allele had increased risk for OM. This association remained significant after adjustment for age and sex \((P < 0.01)\) (Table 4). The IL-4 –1098 and –590 polymorphisms were in linkage disequilibrium in both diseased and control groups \((P < 0.01)\).

It has been shown that IL-4 –590-C/T and –1098-G/T polymorphisms affect gene expression, which is higher when the T allele is present (15). We observed, for the first time to our knowledge, that individuals with the CC genotype at IL-6 –174 G/C had a sevenfold higher risk for OM (Table 4). Very interestingly, we observed an association between CC genotype and the type of microorganism (staphylococci and gram-negative bacteria, \(P < 0.001\)) suggesting that homozygocity for the C allele for IL-6 –174 G/C confers to individuals an increased susceptibility to microbial infections, possibly modifying their inflammatory response to microorganisms. It has been suggested that IL-6 is implicated in OM pathogenesis as it influences osteoclast function and stimulates bone resorption (12).

IL-6 expression has been shown by in vivo, in vitro, and tissue explant studies to be upregulated in the periprosthetic interface loosening membrane (6). However, the role of IL-6 –174 G/C polymorphism on IL-6 production remains unclear. No further associations were found between patients' clinical characteristics (number of the surgical operations, hospitalization days, or readmissions), disease status, type of microorganism, and the rest of the polymorphisms examined.

Combined genotypes in terms of carriers of the risk allele were evaluated and showed an overall association \((P < 0.01)\) (Table 5) (17).

### TABLE 1. Microorganisms isolated from 81 patients with chronic OM

| Isolate type                  | No. of patients | % of patients with isolate |
|-------------------------------|-----------------|---------------------------|
| Gram-negative bacteria        | 14              | 17.3                      |
| Staphylococci                 | 27              | 33.3                      |
| Polymicrobial infections      | 21              | 25.9                      |
| Negative culture              | 19              | 23.5                      |
| Total                         | 81              | 100                       |

### TABLE 2. Minimum detectable ORs under the log additive model

| SNP                  | Group | Minimum detectable OR |
|----------------------|-------|-----------------------|
| IL-1α –889 G/T       | All   | 3.67                  |
| IL-4 –1098 G/T       | All   | 4.11                  |
|                       | –590 C/T | 6.1                    |
| IL-6 –174 G/C        | All   | 2.98                  |

*The minimum detectable OR under the log additive model with a power of 95% and a significance level of 5% was calculated for each comparison.*

### TABLE 3. Association between IL-1α –889-C/T, IL-4 –1098-G/T and –590-C/T, and IL-6 –174-G/C genotype distributions and chronic OM

| SNP and genotype | Patients (\(n = 81\)) | Control subjects (\(n = 110\)) |
|------------------|------------------------|-------------------------------|
| IL-1α –889 C/T   |                        |                               |
| CC               | 42 (51.8)              | 86 (78.2)                     |
| TC               | 28 (34.6)              | 21 (19.1)                     |
| TT               | 11 (13.6)              | 3 (2.7)                       |
| IL-4 –1098 G/T   |                        |                               |
| GG               | 9 (11.1)               | 80 (72.7)                     |
| GT               | 62 (76.6)              | 28 (25.5)                     |
| TT               | 10 (12.3)              | 2 (1.8)                       |
| −590 C/T         |                        |                               |
| CC               | 23 (28.4)              | 88 (80)                       |
| CT               | 51 (63)                | 21 (19.1)                     |
| TT               | 7 (8.6)                | 1 (0.9)                       |
| IL-6 –174 G/C    |                        |                               |
| GG               | 35 (43.2)              | 67 (61)                       |
| GC               | 29 (35.8)              | 38 (34.5)                     |
| CC               | 17 (21)                | 5 (4.54)                      |

*\(P < 0.01\) for all comparisons.*

Overall, it could be suggested that genetic contribution to OM susceptibility could be due to the cumulative effects of several polymorphisms in genes involved in the immune response. However, in genetic associations there is the possibility

### TABLE 4. Unadjusted and adjusted ORs and their corresponding 95% CIs for the association between genotype contrasts and chronic OM

| SNP and genotype(s) | Unadjusted OR (95% CI) | Adjusted for age and sex OR (95% CI) |
|---------------------|------------------------|--------------------------------------|
| IL-1α –889          |                        |                                      |
| CT                  | 2.73 (1.39–5.36)       | 2.14 (0.98–4.24)                     |
| TT                  | 7.51 (1.99–28.36)      | 6.38 (1.67–26.32)                    |
| CT + TT             | 3.33 (1.78–6.24)       | 2.87 (1.21–5.73)                     |
| IL-4 –1098 G/T      |                        |                                      |
| GT                  | 19.68 (2.66–44.73)     | 14.99 (6.19–36.35)                   |
| TT                  | 44.44 (8.39–235.4)     | 31.85 (5.35–189.8)                   |
| GT + TT             | 21.33 (9.49–47.96)     | 16.36 (6.92–38.70)                   |
| −590                |                        |                                      |
| CT                  | 9.29 (4.69–18.43)      | 7.62 (3.65–15.92)                    |
| TT                  | 26.78 (3.14–228.8)     | 17.29 (1.57–191.0)                   |
| CT + TT             | 10.09 (5.15–19.75)     | 8.19 (3.95–16.96)                    |
| IL-6 –174           |                        |                                      |
| GC                  | 1.46 (0.78–2.75)       | 1.12 (0.55–2.28)                    |
| CC                  | 6.51 (2.29–19.12)      | 5.72 (1.76–18.55)                   |
| GC + CC             | 2.05 (1.14–3.67)       | 1.56 (0.81–3.01)                    |

*The minimum detectable OR under the log additive model with a power of 95% and a significance level of 5% was calculated for each comparison.*
that the polymorphisms being tested are not the causal agents, but rather markers of carrier status for the true causal variant by linkage disequilibrium, located nearby. To limit this possibility, we are continuing our study by testing adjacent markers in IL-1α, IL-4, and IL-6 as part of multimarker haplotypes. Additionally, as over 50% of our patients had comorbidities that independently skew the population toward infectious complications, one or more of the studied polymorphisms might be more closely associated with one of these conditions, rather than purely related to OM.

In conclusion, IL-1α −889-C/T, IL-4 −1096-G/T and −590-C/T, and IL-6 −174-G/C polymorphisms are most likely to play an important role in OM pathogenesis. However, as our findings are novel, further studies are needed to delineate the effect of the above polymorphisms in the etiology of OM.

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### TABLE 5. Combined genotypes for patients and controls

| Combined genotype [IL-1α (−889/C/T), IL-4 (−1096/G/T), IL-6 (−174/G/C)]a | % of patients or controls with genotype |
|---|---|
| T carrier, T carrier, T carrier, C carrier | 22.2 0 |
| T carrier, T carrier, T carrier, non-C carrier | 11.1 2.7 |
| T carrier, T carrier, non-T carrier, C carrier | 7.4 0 |
| T carrier, T carrier, non-T carrier, non-C carrier | 2.5 0.91 |
| T carrier, non-T carrier, T carrier, C carrier | 2.5 0 |
| T carrier, non-T carrier, non-T carrier, C carrier | 0 6.4 |
| T carrier, non-T carrier, non-T carrier, non-C carrier | 2.5 11.8 |
| Non-T carrier, T carrier, T carrier, C carrier | 12.4 4.6 |
| Non-T carrier, T carrier, T carrier, non-C carrier | 17.3 9.1 |
| Non-T carrier, T carrier, non-T carrier, C carrier | 6.2 5.5 |
| Non-T carrier, T carrier, non-T carrier, non-C carrier | 9.9 4.6 |
| Non-T carrier, non-T carrier, T carrier, C carrier | 6.2 1.8 |
| Non-T carrier, non-T carrier, T carrier, non-C carrier | 0 1.8 |
| Non-T carrier, non-T carrier, non-T carrier, C carrier | 0 20.9 |
| Non-T carrier, non-T carrier, non-T carrier, non-C carrier | 0 30 |

* Identical and contrasting residues relative to the residues in carriers of the risk allele are shown.