Identification of IL-7 as a candidate disease mediator in osteoarthritis in Chinese Han population: a case-control study

Hong-xin Zhang¹, Yan-gui Wang², Shun-yuan Lu¹, Xiong-xiong Lu³ and Jie Liu⁴

Abstract

Objectives. Little is known about the biochemical mediators IL-7 that correlate with the initiation and progression of OA. We performed this study to assess the role of variants of IL-7 in OA susceptibility in the Chinese Han population.

Methods. We performed a retrospective, case-control study in the Chinese Han population from 2013 to 2015. Four single nucleotide polymorphisms were genotyped (using a ligase detection reaction) in 602 patients and 454 controls. Differences between groups were analysed, and association was assessed by the odds ratio (OR) and 95% CI.

Results. Among these polymorphisms, rs2583764, rs2583760 and rs6993386 showed no significant association with OA in the Chinese Han population (rs2583764 [P-allele = 0.98651, P-genotype = 0.40392, OR (95% CI): 1.00162 (0.83066, 1.20775)]; rs2583760 [P-allele = 0.38450, P-genotype = 0.58752, OR (95% CI): 0.69859 (0.30996, 1.57449)]; rs6993386 [P-allele = 0.69525, P-genotype = 0.50712, OR (95% CI): 0.96432 (0.80406, 1.15653)]). However, the results showed that the rs2583759 polymorphism was significantly associated with OA [P-allele = 0.00 P-genotype = 3.86 x 10⁻³⁻⁰, OR (95% CI): 0.27794 (0.22407, 0.34476)], even when the 10 000 times permutation was performed (P-allele-permutation < 0.00010, P-genotype-permutation = 0.00010). Haplotype analyses showed A-G-A-C, A-G-A-T and G-G-G-C of rs2583764-rs2583760-rs6993386-rs2583759 were risk factors for OA, both before or after the 10 000 times permutation, indicating IL-7 to be associated with OA.

Conclusion. There was a significant association between IL-7, especially rs2583759, and OA in the Chinese Han population.

Key words: osteoarthritis (OA), interleukin-7 (IL-7), single nucleotide polymorphisms (SNPs).

Introduction

OA, which has an important genetic component associated with its development and is an idiopathic phenomenon, occurring in previously intact joints, with no apparent initiating factor such as joint injury or developmental abnormalities, is the most common joint disorder, and many genes contribute modestly to the risk for developing OA [1, 2]. This disease is characterized by the progressive failure of the extracellular cartilage matrix,
leading to articular cartilage destruction [3, 4]. To date, there is no effective treatment for this disease. Current treatments mainly focus on relieving symptoms and improving function, and even joint replacement surgery for end-stage disease. Its incidence is steadily increasing and results in a huge economic burden worldwide. Although the detailed aetiology is not currently fully understood, it is believed that OA develops from an imbalance between anabolic and catabolic processes or homeostasis of cartilage metabolism [5–7].

Biochemical mediators, such as cytokines and growth factors, profoundly influence the cellular responses in joint tissues, modifying both the catabolic and anabolic activities involved in the pathogenesis of OA [8]. ILs, both pro- and anti-inflammatory, have a pivotal role in arthritic diseases and are potential targets of OA therapy [9, 10]. The IL-7 gene is located on chromosome 8q12–q13, spanning 72 kb, and harbours six coding exons. It encodes a 177-amino-acid protein with a 25-amino-acid-long signal peptide. IL-7 is a non-redundant cytokine, essential for T cell survival and development in humans [11]. Studies have indicated that IL-7 is involved in a number of diseases, such as leukaemia, lymphoma, autoimmune diseases and some types of solid tumours [12]. It has been suggested that IL-7 contributes in an autocrine manner to joint tissue destruction in joint diseases such as OA. Long et al. [13] have demonstrated that IL-7 protein is produced by articular chondrocytes, and endogenous production of IL-7 by cartilage tissue is higher when obtained from patients with OA. Miyaura et al. [14] have demonstrated the strong potential of IL-7 for leading to bone loss, and IL-7R-deficient mice displayed increased bone volume and bone density. In this study, to investigate the role of IL-7 in OA, we genotyped four single nucleotide polymorphisms (SNPs) (rs2583764, rs2583760, rs6993386 and rs2583759) of the IL-7 gene in 602 OA patients and 454 normal controls of Chinese Han origin and analysed their association.

Materials and methods

The methods were carried out in accordance with the approved guidelines.

Participants

The sample set consisted of 602 knee OA cases (159 males and 443 females) and 454 normal controls (260 males and 194 females) of the Chinese Han population recruited from the Department of Orthopaedics of Yantai Children’s Hospital. The mean age of the OA cases was 64.5 (9.5) years, and the mean age of the normal controls was 58.5 (11) years. All patients were diagnosed by senior physicians based on standard clinical, endoscopic, radiologic and histological data, on the basis of two criteria: radiographic evidence of disease (defined as a Kellgren–Lawrence grade ≥ 2); and/or clinical evidence of disease requiring total joint replacement (TJR). Patients with other types of arthritis, skeletal dysplasia or tumour were excluded from the study. Controls were randomly selected from healthy persons under routine health screening. The full study conformed to the Declaration of Helsinki and was approved by the Research Ethics Committee of Yantai Children’s Hospital, Yantai, China. All participants provided written informed consent before blood sampling.

SNP selection

We consulted the dbSNP and HapMap databases and selected tag SNPs (using the software Haploview 4.1) with minor allele frequency ≥ 0.2 and r² ≥ 0.5 in the Han Chinese population in Beijing. In total, four SNPs were chosen (rs2583764, rs2583760, rs6993386 and rs2583759) for genotyping. These SNPs span the region where most exons are located. The markers are all intronic SNPs (the location of these SNPs and exons of IL-7 are displayed in supplementary Fig. S1, available at Rheumatology Online). We tested our SNP variability by using a web tool provided by the Broad Institute. By performing this test, our markers could capture 51% of the variability in the whole region.

Genotyping

Genomic DNA was isolated from EDTA-collected peripheral blood using a QIAamp blood extraction kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. All DNA samples were genotyped for IL-7 SNPs using allelic-specific multiple ligase detection reactions according to the standard protocol, and this was carried out by the Shanghai Generay Biotech Co. Ltd. To test the validity of this procedure, ~10% of samples were then confirmed by direct DNA sequencing.

Statistical analysis

In this case–control study, Hardy–Weinberg equilibrium and linkage disequilibrium were calculated by SHEsis software [15, 16]. Allele and genotype frequencies were analysed using gplink (version 2.050). Haplotype frequencies were calculated initially on UNPHASED, then the P-values of the positive haplotype were corrected using the 10,000 permutations on Haploview 4.0RC1. The power of the SNPs in the study was calculated using G Power 3.0.5. The odds ratio (OR) was set at 1.25. All tests were two-tailed and statistical significance was assumed at P < 0.05.

Results

From the linkage disequilibrium plot constructed of the four SNPs (supplementary Fig. S2, available at Rheumatology Online), we found that deviation from the Hardy–Weinberg equilibrium was absent for all cases and controls. The allele and genotype frequencies of the four SNPs in the cases and the healthy controls are listed in Table 1. The haplotype analyses of the cases and the healthy controls are shown in Table 2. In terms of association analysis, we found that the rs2583759 polymorphism was significantly associated with OA [P-allele = 0.00, P-genotype = 3.86 × 10⁻³⁰, OR (95% CI): 0.27794...
Table 1: Allele and genotype frequency of IL-7 in OA

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Alleles</th>
<th>OR (95% CI)</th>
<th>P-values</th>
<th>P-permutation (10 000 times)</th>
<th>Genotypes (frequency)</th>
<th>HWe P</th>
<th>P-values</th>
<th>P-permutation (10 000 times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2583764 A (freq) G (freq)</td>
<td>Case 830 (0.693) 368 (0.307) 1.001615 (0.830663, 1.207749) 0.986510 1.000000 A/A (freq) A/G (freq) G/G (freq)</td>
<td>296 (0.494) 238 (0.397) 65 (0.109) 0.403924 0.996400</td>
<td>Control 626 (0.682) 278 (0.308) 215 (0.476) 196 (0.434) 41 (0.091) 0.699841</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G (freq) C150</td>
<td>Case 1181 (0.986) 17 (0.014) 0.698587 (0.309956, 1.574493) 0.384487 0.994100</td>
<td>583 (0.973) 15 (0.025) 1 (0.002) 0.830700 1.000000</td>
<td>Control 995 (0.990) 9 (0.010) 443 (0.980) 9 (0.020) 0 (0.000) 0.587516 0.999500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6993386 A (freq) G (freq)</td>
<td>Case 780 (0.651) 418 (0.349) 0.964324 (0.804061, 1.156530)</td>
<td>695245 1.000000</td>
<td>Control 796 (0.659) 308 (0.341) 215 (0.476) 208 (0.460) 50 (0.111) 0.605174</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (freq) T (freq)</td>
<td>Case 159 (0.133) 1039 (0.867) 0.277936 (0.224068, 0.344755) 0.00</td>
<td>&lt;0.00001</td>
<td>Control 321 (0.355) 583 (0.645) 197 (0.436) 0.064285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold numbers represent P-values (P < 0.05). *Represents significant positive results. rs2583764-rs2583759 Global result: Global chi-square is 156.695374, while degrees of freedom (df) = 1 (frequency < 0.03 in both control and case has been dropped). Pearson’s P-value is 0.0000. Permutation P-value (Pearson) is 0.0000. rs2583764-rs2583760-rs6993386-rs2583759 Global result: Global chi-square is 291.831390, while df = 5 (frequency < 0.03 in both control and case has been dropped). Pearson’s P-value is 0.00. Permutation P-value (Pearson) is 0.0000. A: Adenine; T: Thymine; C: Cytosine; G: Guanine.

Table 2: Haplotype analysis of the rs2583760-rs2583759 block and the rs2583764-rs2583760-rs6993386-rs2583759 block

<table>
<thead>
<tr>
<th>Group</th>
<th>Haplotype</th>
<th>Case (freq), %</th>
<th>Control (freq), %</th>
<th>(\chi^2)</th>
<th>P-value</th>
<th>P-permutation (10 000 times)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2583760-rs2583759</td>
<td>G C*</td>
<td>143.12 (0.119) 314.24 (0.348)</td>
<td>156.695 0.00</td>
<td>&lt;0.0001</td>
<td>0.255 (0.204, 0.318)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A G A C*</td>
<td>17.40 (0.015) 182.01 (0.201)</td>
<td>209.933 0.00</td>
<td>&lt;0.0001</td>
<td>0.058 (0.035, 0.096)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A G A T*</td>
<td>699.83 (0.584) 386.09 (0.427)</td>
<td>52.739 3.97 x 10^-13</td>
<td>1.00 x 10^-44</td>
<td>1.925 (1.612, 2.299)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G C*</td>
<td>10.76 (0.009) 10.66 (0.012)</td>
<td>8.112 0.004412</td>
<td>0.2570</td>
<td>1.673 (1.170, 2.391)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A G T</td>
<td>54.87 (0.046) 20.80 (0.023)</td>
<td>7.730 0.000544</td>
<td>0.1263</td>
<td>2.040 (1.222, 3.406)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G G C*</td>
<td>17.76 (0.015) 77.86 (0.086)</td>
<td>60.373 8.22 x 10^-16</td>
<td>0.0001</td>
<td>1.159 (0.094, 0.269)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G G T*</td>
<td>272.53 (0.227) 164.34 (0.182)</td>
<td>6.616 0.010125</td>
<td>0.2943</td>
<td>1.329 (1.070, 1.651)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T A T</td>
<td>0.00 (0.000) 1.10 (0.001)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G T G C</td>
<td>15.95 (0.013) 7.90 (0.009)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A T A T</td>
<td>1.01 (0.001) 0.00 (0.000)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G T A C</td>
<td>0.03 (0.000) 0.00 (0.000)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold numbers represent P-values (P < 0.05). *Represents significant positive results. rs2583760-rs2583759 Global result: Global chi-square is 156.695374, while degrees of freedom (df) = 1 (frequency < 0.03 in both control and case has been dropped). Pearson’s P-value is 0.0000. Permutation P-value (Pearson) is 0.0000. rs2583764-rs2583760-rs6993386-rs2583759 Global result: Global chi-square is 291.831390, while df = 5 (frequency < 0.03 in both control and case has been dropped). Pearson’s P-value is 0.00. Permutation P-value (Pearson) is 0.0000. A: Adenine; T: Thymine; C: Cytosine; G: Guanine.
(0.22407, 0.34476). After 10,000 permutations correction, rs2583759 was still significant in the allele distribution (P-allele-permutation < 0.00010, P-genotype-permutation = 0.00010).

Haplotype analyses revealed that the G-C and G-T haplotypes consisting of rs2583760–rs2583759 were positively associated with OA [G-C: $\chi^2 = 156.695$, $P < 0.00010$, P-permutation < 0.00010, OR (95% CI): 0.255 (0.204, 0.318); G-T: $\chi^2 = 156.695$, $P < 0.00010$, P-permutation < 0.00010, OR (95% CI): 3.924 (3.142, 4.900)], showing that they were risk factors for OA. And A-G-A-C, A-G-A-T, A-G-G-C, G-G-A-T and G-G-G-C and G-G-G-T of rs2583764–rs2583760–rs6993386–rs2583759 were also risk factors for OA before the 10,000 times permutation [A-G-A-C: $\chi^2 = 209.933$, $P = 0.00$, OR (95% CI): 0.058 (0.035, 0.096); A-G-A-T: $\chi^2 = 52.739$, $P = 3.97 \times 10^{-13}$, OR (95% CI): 1.925 (1.612, 2.299); A-G-G-C: $\chi^2 = 8.112$, $P = 0.004412$, OR (95% CI): 1.673 (1.170, 2.391); G-G-A-T: $\chi^2 = 7.730$, $P = 0.005444$, OR (95% CI): 2.040 (1.222, 3.406); G-G-G-C: $\chi^2 = 60.373$, $P = 8.22 \times 10^{-15}$, OR (95% CI): 0.159 (0.094, 0.269); G-G-G-T: $\chi^2 = 6.616$, $P = 0.010125$, OR (95% CI): 1.329 (1.070, 1.651)]. However, when the 10,000 times permutation was performed, A-G-G-C, G-G-A-T and G-G-G-T no longer showed significant association (A-G-A-C: P-permutation = 0.2570; G-G-A-T: P-permutation = 0.1263; G-G-G-T: P-permutation = 0.2943).

Discussion

IL-7 is a potent immune-regulatory cytokine belonging to the IL-2/IL-15 family. It is produced by various cell types, including stromal cells, endothelial cells, epithelial cells, osteoclasts, chondrocytes, fibroblasts, smooth muscle cells and even some malignant cells [17]. IL-7 is well known for its critical role in the development and homeostasis of lymphocytes [18]. However, accelerating evidence is revealing that IL-7 is involved in other pathologies, such as tumour development and progression, bone biology and joint diseases [12, 17, 19]. Studies from Richard F. Loeser’s group have indicated that IL-7 is associated with OA, and our study is the first research to do so. We tested three SNPs (rs2583764, rs2583760 and rs6993386) between exons 2 and 3, and one SNP (rs2583759) between exons 5 and 6. From the results of both allele analyses and genotype analyses, we concluded that IL-7, especially rs2583759, is significantly associated with OA. The linkage disequilibrium blocks (supplementary Fig. S2, available at Rheumatology Online) showed that rs2583759 has a hot relationship with rs2583760, which might be critical in the development of OA. Besides, the SNPs rs2583764, rs2583760 and rs6993386 are in the same block. Further analyses revealed that these SNPs did have linkage disequilibrium. This study represents the first attempt to test the association between polymorphisms in the IL-7 gene and OA. We recruited 602 patients and 454 controls and used four SNPs as tag markers to investigate the role of IL-7 in OA patients of Chinese Han origin. Our data indicated that rs2583759 is probably associated with OA and that IL-7 may be a potential risk gene for OA in the Chinese Han population. However, the present study is only based on subjects of the Chinese Han population and the genotyped four tag SNPs—replicating studies with larger samples and with more markers in other ethnic groups will be necessary to clarify the apparent relationship of IL-7 to OA. Furthermore, research into the functional role that IL-7 plays in the pathology of OA is needed, and this would provide further useful information about prevention, diagnosis and therapy of this disease.

Acknowledgements

Author Contributions: J.L. and H.Z. designed and managed the research work. Y.W. and X.L. improved the manuscript. H.Z. and S.L. performed the experiments. J.L. and H.Z. analysed the data and wrote the manuscript.

Funding: This work is supported by grants from the National Natural Science Foundation of China (grant number 31000408, 81401823), the Science and Technology Commission of Shanghai municipality (13ZR1461100, 14ZR1425500), the Shanghai Municipal Bureau of Health for Young Researchers (grant number Y123), the Science and Technology Fund Projects from Shanghai Jiao Tong University School of Medicine (13XJ10059), the Science and Technology Development Plan of Yantai City (2013WS243), the Young Teacher Training Scheme from Shanghai Universities and the Outstanding Young Teacher Training Scheme from Ruijin Hospital.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

References


10 Rübenhagen R, Schuttrumpf JP, Stürmer KM, Frosch KH. Interleukin-7 levels in synovial fluid increase with age and MMP-1 levels decrease with progression of osteoarthritis. Acta Orthop 2012;83:59–64.