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Association of interleukin-6 gene polymorphisms with hand osteoarthritis and hand osteoporosis

Orit Blumenfeld [a], Frances M.K. Williams [b], Ana Valdes [b], Deborah J. Hart [b], Ida Malkin [a], Timothy D. Spector [b], Gregory Livshits [a,b,*]

[a] Human Population Biology Research Unit, Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[b] Department of Twin Research and Genetic Epidemiology, King's College London St Thomas' Hospital Campus, London, UK

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ABSTRACT

Objective: Several genes, including *IL-6* encoding pro-inflammatory cytokines, are involved in development of osteoarthritis and osteoporosis. The association of radiographic hand osteoarthritis (RHOA) and osteoporosis related phenotypes (RHOP) with polymorphisms in *IL-6* has been reported inconsistently. The aim of this study was to examine the association, between RHOA and RHOP and *IL-6* polymorphisms in two independent samples.
Methods: Two samples: UK females, including 1440 individuals assessed for RHOA and 3470 assessed for RHOP; Chuvash pedigree including 1499 females and males were assessed for RHOP and RHOA. SNPs were genotyped in the IL-6 genomic region, and used in association analysis with RHOA and RHOP phenotypes.
Results: RHOP phenotypes showed similar heritability estimates in both samples, ranging from 34.5 ± 5.5% to 61.0 ± 2.4%. RHOA in Chuvash had substantially lower heritability estimates compared to twins (e.g. OSP scores: 11.8 ± 2.3% vs. 39.2 ± 4.1%) with much higher prevalence and considerably stronger correlation with age ($r = 0.811$ vs. $r = 0.505$). RHOA in Chuvash sample may be traumatic in nature, caused by heavy and prolonged manual work related to their private farming. There were a number of statistically significant association results with both types of phenotypes. The most consistent result was obtained for JSN in both samples with SNP from the same haploblock. Their combined probability of no association was only $p = 0.000003$. Additionally, there were SNPs common for both RHOA and RHOP.
Conclusions: We have shown polymorphisms in *IL_6* are significantly associated with RHOA and hand RHOP in two samples having different ethnicity and lifestyle. Age × environment × genes interaction appears as an important factor of RHOA manifestation and progression.

1. Introduction

Osteoporosis and osteoarthritis are two major and most common musculoskeletal disorders. Although both conditions have a multifactorial nature, they develop differently, have different symptoms, and are diagnosed differently. Both conditions, however, governed to a large extent by genetic factors and involve inflammatory processes. A major contribution of genes to appearance of osteoporosis-related phenotypes, bone mass loss and others, as well as to a development and manifestation of osteoarthritis, is well established [1,2]. However, specific genetic factors and polymorphisms associated with these conditions remain poorly understood, and numerous candidate genes and genomic regions have been proposed [3–6]. The interleukin-6 gene

Abbreviations: AD, additive; B_AREA, bone area; BG, bone geometry; BL, bone length; BMD, bone mineral density; BMD_C, bone mineral density, compact compartment; BMD_UR, bone mineral density at ultra-distal radius; BMD_UU, bone mineral density at ultra-distal ulna; BMD_T, bone mineral density_total; BMI, body mass index; CE, common family environment; COV, covariates; GM, general model; HS, household; IL-6, interleukin – 6; JSN, joint space narrowing; K/L, Kellgren/Lawrence grading scale; MCI, metacarpal cortical index; MPM, most parsimonious model; OSP, osteophytes; RHOA, radiographic hand osteoarthritis; RHOP, radiographic hand osteoporosis; RS, residual; SB, siblings; SNP, single nucleotide polymorphism; SP, spouse.

* Corresponding author at: Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel. Tel.: +972 3 6409494; fax: +972 3 6408287.
E-mail address: gregl@post.tau.ac.il (G. Livshits).

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(*IL-6*) has been proposed as a candidate gene for a variety of osteoporosis and osteoarthritis phenotypes because of its role in inflammation, but studies have reported quite contradictory results [7,8]. At least 50 single nucleotide polymorphisms (SNP) and five common haplotypes have been identified in *IL-6*, and have been surveyed recently [9,10]. The epidemiological data suggest that circulating IL-6 is a significant factor in bone loss [11], as well as in a development of osteoarthritis-related phenotypes [12]. However, testing the genetic association between *IL-6* variants and osteoarthritis and osteoporosis phenotypes assessed on lower and upper limb bones and joints, has generated inconsistent results. While some studies found statistically significant associations [13–18] others – did not [16,19–21]. Thus, the question concerning the association between osteoarthritis – and osteoporosis-related phenotypes variations with genetic variation of *IL-6* remains largely unresolved.

It should be mentioned that manifestation of radiographic hand osteoarthritis (RHOA) and osteoporosis (RHOP) – related phenotypes may not be independent for several reasons. One of the RHOA characteristics is that it is associated with presence of hand osteophytes and cartilage loss [22], but the progress in RHOA is correlated with hand bone mineral density (BMD) [23]. The link between BMD and RHOA has been explained by pleiotropy, i.e. shared genetic effects [24]. The potential mechanism for this is involvement of inflammatory processes in development of both osteo- porosis and –arthritis. Osteoarthritis is characterized by chronic local inflammation of the joints involving pro-inflammatory mediators, including IL-6 that causes cartilage degradation by upregulation of catabolic factors [25]. On the other side, IL-6 is also involved in bone remodeling [9], i.e. RHOP could also be dependent on IL-6 action and genetics. Thus, testing the hypothesis that genetic variation in *IL-6* variants influence RHOA and RHOP is of interest and importance. In the present study we examined these associations using two independent samples.

2. Material and methods

2.1. Samples

1. *TwinsUK register*. The present study is based on 1440 individuals, assessed for RHOA, and 3470 individuals assessed for bone mineral density at ultradistal radius and ulna (BMD_UR & BMD_UU, respectively). The Twins UK Adult Twin Registry, described in detail elsewhere [26], now includes >12,000 volunteer participants. Twin participants in this study gave written informed consent and the St. Thomas' Hospital research ethics committee had approved the project. The register has been collected from the general population through national media campaigns in the UK without first ascertaining the presence of any individual characteristics, diseases or traits. Collected DNA was sent for genome wide genotyping using the Illumina (San Diego, USA) 317 K and 610 K SNP arrays [27]. The age and trait description of the sample is given in Table 1 for RHOA and RHOP phenotypes separately. As the cohort is overwhelmingly of North European ancestry (98%), participants of other ethnicities were not included in the current study.
2. *The Chuvash*. This sample includes Caucasian individuals (descendants of Bulgar tribes) and has been described elsewhere [28]. The sample consists of 269 nuclear families, including 782 men and 717 women, with age range 17–90 years. All individuals in the sample were assessed for RHOA and for several RHOP phenotypes. The pedigrees were collected randomly, and included individuals having no chronic infection, nor metabolic or bone-related diseases. All subjects who agreed to participate in the study signed an informed consent form and the Tel Aviv University ethics committee had approved the project. Of 1499 individuals 1100 individuals were genotyped for 4 SNPs in *IL-6* genome region.

2.2. RHOA and BS related phenotypes

(A) The RHOA was assessed using similar methodology in both samples, by plain posterior–anterior radiographs, taken from both hands of each study participant. The films from twins were not read as pairs. The radiographic features of hand OA, including the presence of osteophytes (OSP), joint space narrowing (JSN) and Kellgren/Lawrence grades, were scored for each of the 14 joints on both hands. OSP and JSN were separately evaluated and graded from 0 to 3 for increasing severity using a standardized atlas. The summary K/L grade for each joint and in total was evaluated from 0 to 4 following the original atlas [29].
(B) RHOP phenotypes. In TwinsUK measurements of BMD_UR and BMD_UU, located proximal to the radial and ulnar end plates correspondingly were assessed using Hologic QDR-2000 DXA scanner as detailed in [30].

In the Chuvash sample, standard plain radiographs of both hands were taken in the postero-anterior position from each participating individual. The detailed description of the procedure is given elsewhere [31]. Measurements on digitalized radiographs were carried out by means of UTHSCSA Image Tool Version 3.0 for Windows software package (http://ddsdx.uthscsa.edu/dig/itdesc.html) using the scripts written by I. Malkin specifically for this purpose. The measurements included bone mineral density of the total bone (BMD_T) and its compact compartment (BMD_C), and bone surface area (B_AREA). The corresponding measurements were taken from the radiographic images of the second to fourth fingers on each of the metacarpal bone, proximal and middle phalanges, 18 bones in total [32]. In addition, we measured the metacarpal cortical index (MCI = CWT/D), representing the ratio of the cortical bone wall compact layer thickness (CWT) to bone total diameter (D) on 6 metacarpal bones [33].

2.3. SNP selection

2.3.1. TwinsUK

The genotype data were based on genome-wide genotyping scans performed in this cohort previously using the Illumina (San Diego, USA) 317 K and 610 K SNP arrays, with a call rate of genotype $\geqslant$ 98%. Using the published data, the International HapMap and UCSC browsers, the *IL-6* region was identified and positioned between 22,727,147 and 22,732,002 bp on chromosome 7q21. Fifteen genotyped SNPs were available in this region and close to it (22,702,977–22,754,799 bp), in our sample. These SNPs covered the entire *IL-6* locus and some markers were also located approximately 24Kbps up- and 23Kbps down-stream of the structural gene. All SNPs were at Hardy–Weinberg equilibrium $p > 0.05$ and with minor allele frequency (MAF) ranging between 0.11 and 0.47 (Table S1, Supplementary material).

2.3.2. Chuvash sample

Using Nucleon BACC Genomic DNA Extraction Kits (Amersham International plc, UK), DNA was prepared from peripheral blood lymphocytes by standard techniques, according to the manufacturer's protocol. The selected four SNPs were genotyped in 1090 individuals by KBiosciences (Hertsfordshire, UK) using their proprietary competitive allele-specific PCR (KASPar) method (the details are available at the company's website (www.kbioscience.co.uk/chemistry/index.htm). The genotyped SNPs were mapped at region 22,724,028–22,746,443, and were all at