GENETIC INVESTIGATION OF KNEE OSTEOARTHRITIS

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INTRODUCTION:

Osteoarthritis is a common disease characterized by the degeneration of the cartilage of synovial joints such as the hip and knee, leading to bone changes such as osteophytosis, subchondral sclerosis and formation of bony cysts. It is a complex, multifactorial disorder in which it has been shown that genetic factors play an important role in its etiology. Given the essential role of sex steroid action in bone metabolism and homeostasis, genes encoding estrogen and androgen receptors are considered important candidates for the determination of osteoarthritis risk. Estrogens affect articular cartilage metabolism directly via estrogen receptors (ER) in chondrocytes. To elucidate the possible role of genetic variation in the estrogen receptors α and β (ER-α, ER-β) and androgen receptor (AR) genes with knee osteoarthritis, the -1174(TA)n, c.1092+3607(CA)n, and c.172(CAG)m repeat polymorphisms of the ER-α and ER-β genes between OA patients and controls (p<0.005 and p<0.0001 respectively). A significantly increased odds ratio for knee OA was observed in individuals having LL genotype for ER-α gene and LL and SL genotypes for ER-β gene compared to individuals with the SS genotype (95% CI 1.03-3.5; p=0.04 and CI 2.4-8.3 and 2.5-7.5; p=0.001 respectively). When odds ratios were adjusted for age, sex, BMI grade of physical demands, age of menarche and menopause all together, it was observed that women with LL genotypes for ER-β gene had a 3-fold increased risk to develop knee OA compared to the ones with SS genotype (95% CI 1.33-7.45; p=0.009). For AR gene, it was also observed that the women with LL genotypes had a significantly increased risk for knee osteoarthritis compared to the ones with SS genotype (OR=0.017, 95% CI 0.001-0.16; p=0.001) . The above statistical significances were retained even after the Bonferroni correction (p=0.01), fact that improves the genetic resolution and strengthens the analysis. For ER-α gene, after the adjustment for the various risk factors, no statistical significance was observed.

METHODS:

(i) For the association study: A case-control cohort of 158 patients with idiopathic knee osteoarthritis and 193 controls (who had undergone treatment for injuries and fractures) were used. Genomic DNA was isolated and appropriate primers were designed for PCR amplification. All forward primers were fluorescently Cy5.0 labeled and allele fragment sizes were determined in comparison with external size markers by an automated DNA sequencer and analyzed using the Fragment Analysis Software. All alleles were divided into two groups of approximately equal size; those with short(SS)and those with long alleles(LL). For statistical analysis unadjusted (univariate) OR for various genotypes was estimated and subsequently included in the logistic regression models variables known to be associated with OA (age, sex, BMI, age at menarche and menopause and grade of physical demands). The Monte Carlo test was used in order to further investigate the association between OA and alleles at highly polymorphic loci. The Monte Carlo test was used in order to further investigate the association between OA and alleles at highly polymorphic loci. The Bonferroni method was additionally applied to the findings from the multivariate logistic regression analysis, in order to strengthen the statistical analysis.

(ii) For linkage analysis: A total of 27 families having at least 2 affected members in each one (108 individuals) were used. The structures of the families participated were: 9 families with affected sibling pairs, 11 families with affected parent-offsprings, 5 families with both affected sibling pairs and parent-offsprings and 2 with affected sibling pairs, parents-offsprings and cousins. Isolation of genomic DNA was performed using a commercially available kit. 19 microsatellite markers were selected. (D6S1695, D6S447, D16S420, D16S407, D6S1579, D6S1717, D6S1712, D16S1303, D6S423, D16S3070, D6S1556, D6S1637, D6S1684, D6S1620, D6S1585, D6S1626, D6S1619, D6S1646, D6S456), 15 for chromosome 6q and 4 for chromosome 16q at a distance of approximately 8 cm. Microsatellite markers were amplified by polymerase chain reaction (PCR) and the amplification products were electrophoresed in an automated DNA sequencer. Allele sizes were determined using the Fragment Analysis Software of a DNA sequencer (Visible Genetics, Inc.) and LOD scores were determined for each marker in each family. All markers were monitored for genotyping errors with independent typing by a second individual.

For both the association and the linkage studies, anterior-posterior weight-bearing knee radiographs – for all the participants- were obtained and assessed according to Kellgren / Lawrence scale by two independent expert observers. Patients with rheumatoid arthritis and other autoimmune diseases as well as chondrodysplasias, infection-induced OA and post-traumatic OA were not included in the studies. The study was approved by the ethics committee of Larissa University Hospital.

RESULTS SECTION:

(i) A significant difference was observed in the frequency distribution of -1174(TA)n and c.1092+3607(CA)n, repeat polymorphisms of the ER-α and ER-β genes between OA patients and controls (p<0.005 and p<0.0001 respectively). A significantly increased odds ratio for knee OA was observed in individuals having LL genotype for ER-α gene and LL and SL genotypes for ER-β gene compared to individuals with the SS genotype (95% CI 1.03-3.5; p=0.01 and CI 2.4-8.3 and 2.5-7.5; p=0.001 respectively). When odds ratios were adjusted for age, sex, BMI grade of physical demands, age of menarche and menopause all together, it was observed that women with LL genotypes for ER-β gene had a 3-fold increased risk to develop knee OA compared to the ones with SS genotype (95% CI 1.33-7.45; p=0.009). For AR gene, it was also observed that the women with LL genotypes had a significantly increased risk for knee osteoarthritis compared to the ones with SS genotype (OR=0.017, 95% CI 0.001-0.16; p=0.001) . The above statistical significances were retained even after the Bonferroni correction (p=0.01), fact that improves the genetic resolution and strengthens the analysis. For ER-α gene, after the adjustment for the various risk factors, no statistical significance was observed.

(ii) LOD scores were < 0.1 for all markers used. The highest LOD score observed was 0.09 for the microsatellite marker D16S3070. From the above microsatellite marker D16S3070 had a negative LOD score (range -0.01 to -0.24) and 4 had a LOD score with a range from 0.02 to 0.09. These findings indicate that there is no association between knee OA and genes located on chromosomes 6q and 16q in the specific Greek population in the district of Thessaly(Central Greece).

DISCUSSION:

Our results indicate that:

(a) Chromosomes 6 and 16 are not likely to harbor OA susceptibility genes in our study population. Previous studies have reported linkage to chromosomes 2, 4, 6, 7, 11, 16 and X. We believe that our results are conflicting, probably because of the existing difference between the forms of knee OA from region to region. Knee OA in the Central Greece region is characterized mainly by many large osteophytes and less by joint space narrowing (JSN). However, we recognize that our linkage analysis study may have insufficient power due to the small number of participants, so larger sample number is needed in order to confirm our conclusions regarding the involvement of chromosomes 6q and 16q in knee O.A

(b) There is a relationship between c.1092+3607(CA)n and c.172(CAG)m, repeat polymorphisms of the ER-β and AR genes and knee osteoarthritis. Since the underlying molecular mechanisms are not yet known, it can be suggested that the above microsatellite repeat polymorphisms might affect levels of expression through transcriptional regulation or by being in linkage disequilibrium with exon alterations that may affect ER or AR protein function, or even by being linked with alterations in unidentified genes adjacent to ER-β or AR genes which may modulate OA risk.

Our studies have several strengths as they included: 1) participants with knee osteoarthritis only and 2) a homogeneous population (Central Greece inhabitants), since if there were any undetected racial / ethnic differences between the cases and controls, apparent associations between particular alleles and the disease could be confounded. Also, the availability of detailed questionnaire and interview information allowed us to consider potential confounding factors. Further studies are needed to explore the finding that the examined microsatellite polymorphisms strongly influence knee osteoarthritis risk and to delineate the potential role of the above repeat polymorphisms in the etiology of knee osteoarthritis.