RESEARCH LETTER

Synonymous SNP influences Adam12 mRNA expression level in synovial tissue

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Several studies have established that in addition to age, sex, body mass index and trauma, genetic background also contributes to the risk of osteoarthritis (OA). ADAM12, one of the main proteolytic enzymes that regulates extracellular matrix turnover in OA joint tissues, is likely to be one of such genes, because genetic association studies have indicated a link between ADAM12 genetic variants and OA susceptibility and progression traits (Valdes et al, 2004). However, the functional impact of ADAM12 polymorphisms in osteoarthritic joint tissues has not yet been studied. Previously, the allele-specific expression of several OA-associated genes (GDF5, DIO2) has been reported in cartilage and other tissue of the synovial joints, emphasizing the need to consider the OA as involving the entire joint (van Meurs and Uitterlinden, 2012). One of the key factors in the OA pathophysiology is inflammation of the synovial membrane, which is associated with risk of progressive cartilage degradation and signs and symptoms of diseases (Sellam and Berenbaum, 2010). We aimed to evaluate the influence of the ADAM12 SNP on mRNA expression in the synovial tissue of patients with early knee OA (KOA).

The synovial tissue samples were harvested in 44 middle-aged subjects (aged 32–60, mean 46.7 years, 24 women) who had undergone arthroscopy due to chronic knee complaints. X-rays of the knee joints were performed on all participants for the estimation of radiographic KOA using the Nagaosa et Doherty grading system (Nagaosa et al, 2000). The expression of ADAM12 mRNA, assessed by 2-ΔΔCT method, was measured in synovial samples by TaqMan® Gene Expression Assay (Hs01106104; Applied Biosystems, Foster City, CA). The synovial tissue harvested from macroscopically intact synovia was used as a control sample.

Four SNPs in the ADAM12 gene (rs3740199, rs1871054, rs1044122, rs1278279) were genotyped in all subjects using TaqMan® SNP Genotyping Assays. The expression of ADAM12 mRNA in synovia was compared with genotypes of investigated polymorphisms in the ADAM12 gene, as well as with detailed phenotypical features of OA (presence of osteophytes, joint space narrowing). The Wilcoxon exact test (WET) was applied for evaluation of the association between expression level (assessed by 2-ΔΔCT) and polymorphisms of the ADAM12 gene.

We found that the synonymous polymorphism rs1278279 (p. c.1515G > A, p.N505N) in exon 14 of the ADAM12 gene influences the overall expression of ADAM12 mRNA in synovial tissue. The rs1278279 genotypes in the study groups are distributed as follows: 36 GG homozygotes and 8 GA heterozygotes. In our study group, GG homozygotes had a lower relative expression level of ADAM12 mRNA in synovia compared to subjects with AG genotype (Wilcoxon exact test; p = 0.03, Figure 1). Separate analyses in men and women did not reveal statistically significant differences in mRNA expression, probably due to the smaller number of subjects in the groups; however, a trend for higher expression in AG heterozygotes was noticed in males (p = 0.06, WET). For other SNPs, no difference was observed in ADAM12 mRNA expression between distinct genotypes. Additionally, there was no association between severity of radiographic KOA and expression level of ADAM12 mRNA.

The rs1278279 is a synonymous polymorphism in exon 14 of the ADAM12 gene, which results in asparagine-coding triplet substitution (AAC→AAT). As our previous data showed rs1278279 and rs1044122 demonstrate strong linkage disequilibrium (LD) across (D’>80%) (Kerna et al, 2013). The same study revealed that rs1044122 carried the higher risk for early knee OA, whereas no statistically significant associations were found for rs1278279. The possible reason for that could be insufficient power of the study regarding detection of associations for rs1278279 with low minor allele frequency (MAF 16%); however, reported associations of several polymorphisms of ADAM12 with...
OA could suggest the putative relation of this gene region to specific pathophysiological pathways of OA.

The mechanism, which could be responsible for regulation of mRNA expression level by rs1278279, is presently unknown. Most frequently, the spectrum of the action by which cis-acting polymorphisms could influence gene expression includes transcriptional control, relative isoform expression, and mRNA stability (Pastinen et al, 2006). Synonymous mutations do not alter the encoded protein, but they can influence gene expression via changes in secondary structures of mRNA and thereby alter the length of pause cycles during translation, the overall rate of translation, or protein folding (Kimchi-Sarfaty et al, 2007; Bartoszewski et al, 2010). Indeed, the ubiquitous long pauses can lead to translational frame shifting and to protein misfolding (Wen et al, 2008). The assumption that triplet AAT (AG heterozygote in our study) may result in shorter pause time during translation could putatively explain higher expression levels of ADAM12 mRNA.

Briefly, our results suggest that synonymous variant rs1278279 in the ADAM12 gene could influence ADAM12 mRNA expression in the synovial membrane of the knee joint. The mechanism of regulation for ADAM12 mRNA and protein expression is currently unclear and needs to be clarified by further investigation. A better understanding of molecular mechanisms of ADAM12 mRNA/protein expression regulation in OA joint tissue could potentially help in the development of new therapeutic approaches in the field of OA.

REFERENCES


