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Association of a nsSNP in ADAMTS14 to some osteoarthritis phenotypes

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Summary

Objective: To investigate the effect in OA (Osteoarthritis) susceptibility of putative damaging changes in ADAM (A Disintegrin And Metalloprotease) and ADAMTS (ADAM with ThromboSpondin motif) proteases.

Methods: Non-synonymous single nucleotide polymorphisms (nsSNP) in 18 ADAMTS and 31 ADAM genes were analyzed with two software applications for prediction of functional damage. Four putative damaging nsSNP were found in ADAMTS2, ADAMTS14, ADAMTS16 and ADAM12, respectively. These nsSNPs were analyzed in case-control sample collections with a variety of phenotypes totalling 3217 OA patients and 2214 healthy controls, all of them Caucasians.

Results: No statistically significant differences were found in ADAMTS2, ADAMTS16 and ADAM12 nsSNPs. Conversely, the rare allele of the rs4747096 nsSNP in ADAMTS14 was overrepresented in women requiring joint replacement because of knee OA ($O.R._{M\text{-}H}$ (odds ratio. Mantel-Haenszel) = 1.41, 95% C.I. = 1.1–1.8; $P = 0.002$) and in patients with symptomatic hand OA (O.R. = 1.37, 95% C.I. = 1.0–1.9; $P = 0.047$). A non significant increase in the frequency of the same allele was also found in patients with hip OA requiring prosthesis ($O.R._{M\text{-}H} = 1.14$, 95% C.I. = 1.0–1.3; $P = 0.08$). No association was found with other OA phenotypes.

Conclusion: Our findings implicate ADAMTS14 in OA, specifically in knee OA requiring joint replacement in women and, possibly, in hand OA. Independent association of ADAMTS14 genetic variation to knee OA in women has been communicated. ADAMTS14 involvement, if confirmed, will open a new area of interest in OA pathogenesis because of its role in the maturation of collagen fibers.

Key words: Genetic susceptibility, Osteoarthritis, Matrix metalloproteases, Non-synonymous polymorphisms.

Introduction

Primary osteoarthritis (OA) is the most prevalent form of arthritis and a cause of important handicap among the elderly. This situation is compounded by our incomplete knowledge of OA molecular mechanisms and etiological factors. The latter are complex, including a wide array of non-genetic factors and a multiplicity of weak genetic factors[1–3]. Some of the specific OA genetic factors have already been identified. Notable examples are polymorphisms in asporin[4], FRZB[5] and GDF5[6]. These three genetic factors are involved in controlling growth and differentiation pathways, asporin by regulating the availability of free TGF-β[7]; FRZB as a soluble inhibitor of Wnt ligands; and GDF5 as a cartilage growth and differentiation factor. Many other genetic variants have shown association to OA but they have not been sufficiently confirmed. Further research is needed to replicate previous findings and to identify new OA genetic factors.

The genes encoding members of the two large families of proteases ADAM and ADAMTS are of interest in OA as possible genetic factors[8]. Proteins of these families share two functional domains, a metalloprotease domain, the active site common to all metalloproteases, and a disintegrin domain that mediates adhesion. In addition, the ADAMTS proteins include thrombospondin motifs. Most ADAMs are membrane-bound proteases and their best known functions are ectodomain shedding and regulation of cell adhesion,

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although they can also have a role in matrix degradation[9,10]. Ectodomain shedding, the proteolytic cleavage of extracellular protein domains, affects growth factors, cytokines and cell adhesion molecules. Some members of the ADAM family can be involved in OA. For example, ADAM8 and ADAM12 are involved in osteoclast differentiation[11,12], ADAM10 is implicated in cell fate determination of osteoblasts[13], ADAM15 enhances chondrocyte adhesion to collagen, promotes chondrocyte survival and protects from experimental OA[14]; and ADAM17 releases TNF-α form its membrane-bound precursor[15]. The ADAMTS family contains all the known aggrecanases (ADAMTS1, -4, -5, -8, -9, -15). These enzymes cut the aggrecan core protein at a particular site that seems critical for cartilage damage[16]. One of them, ADAMTS5 is necessary for cartilage damage in murine models of arthritis[17,18]. However, it is unknown if ADAMTS5 is as crucial in humans as in these models[19]. We have recently shown that genetic variation in this gene seems irrelevant for OA predisposition[20]. In addition, some of the ADAMTS are able to degrade other matrix components apart from aggrecan and three of them, ADAMTS2, -3 and -14, are involved in processing of collagen precursors, procollagen types I, II or III, by cleaving their N-terminal propeptides. Also indicative of the possible relevance of some ADAM or ADAMTS proteases for OA are studies of mRNA expression and of genetic association. Systematic analyses of gene expression by microarray hybridization and specific studies by quantitative PCR (qPCR) have shown a wide array of significant differences between control and OA cartilage[21–24]. In addition, genetic association of an non-synonymous single nucleotide polymorphisms (nsSNP) in ADAM12 to knee OA has been reported[25].

Here, we have examined all the ADAMTS and ADAM members looking for nsSNPs. Those predicted to be damaging for protein function were studied in a variety of OA phenotypes. An nsSNPs in ADAMTS14 showed association to OA in some of them, most notably knee OA requiring joint replacement.

Material and methods

Patients and controls: Six already described sample collections of European Caucasians were used[20,26–30]. They were divided in two sets, a first set included samples with hand OA or with knee or hip OA requiring total joint replacement in which all single nucleotide polymorphisms (SNPs) were analyzed in the Santiago laboratory. The second set included other OA phenotypes in which only the rs4747096 SNP was studied in different laboratories. The first set included samples from three collections, the Hospital Clinico de Santiago in Spain; the Institute of Musculoskeletal Sciences, University of Oxford in the UK; and the Departments of Biology and Genetics and of Orthopaedics, University of Thessaly in Greece. These three collections of samples include knee or hip OA patients that have undergone total joint replacement (total knee replacement (TKR) and total hip replacement (THR) groups, respectively) due to the severity of their lesions. Exclusion of inflammatory, posttraumatic or postseptic arthritis, as well as cases suggestive of dysplasia was performed. The Santiago collection also included patients with hand OA according to the American College of Rheumatology (ACR) classification criteria (HOA; 242 subjects, 213 women, 27 men and 2 unrecorded; mean age 61 years, range 32–88). Other patients of this collection were 307 subjects of the THR group (185 women and 122 men; mean age 68 years, range 55–84) and 262 of the TKR group (211 women and 51 men; mean age 68 years, range 55–80). Patient evaluation included review by a rheumatologist of a specific questionnaire, clinical history and radiographs previous to surgery. The 294 selected controls (115 women and 179 men; mean age 68 years, range 55–94) did not show clinical manifestations of OA (absence of chronic pain or restriction of mobility in the two years before recruitment, no hand enlargements or deformities, and no previous medical evaluation as OA). All of the subjects in this collection were of Caucasian Spanish ancestry. The Oxford's collection was of 1105 THR samples (629 females and 476 males), 360 TKR samples (196 women, 164 men). All patients have a Kellgren/Lawrence (K/L) score of >2 and $>90\%$ had a K/L score of 3 or 4. The average age of patients was 65 years (range 55–85 years). The 698 controls of this collection (356 women, 342 men) had no signs or symptoms of arthritis or joint disease (pain, swelling, tenderness, or restriction of movement). The average age of the controls was 69 years (range 55–89 years). All were UK Caucasians. The Thessaly's collection included 159 TKR patients (139 women with mean age 68 years and range 48–92, and 20 men with mean age 72 years and range 62–85). All of them had K/L score >2 and 92 % had a 3–4 score. The control population consisted of 193 subjects (137 women with mean age 68 years and range 44–87, and 56 men with mean age 70 years and range 46–88), who had no clinical OA and that showed a K/L score of 0. All individuals were of Greek origin living in the district of Thessaly.

The second set was made of three collections: Corunna, Leiden and London. The first included 265 clinically symptomatic and radiographically confirmed knee OA patients (KOA; 222 women and 43 men; mean age 67 years, range 30–96) from the Hospital Juan Canalejo, A Corunna in Spain (this collection did not include controls). All of them were Spanish Caucasians. The Leiden collection was made of 358 subjects with familial OA at multiple sites (292 women and 66 men of Dutch ancestry sib pairs; mean age 60 years, range 43–79) from the Genetics osteoARthritis and Progression (GARP) study. Probands and siblings have OA at multiple joint sites in the hands or OA in two or more of the following joint sites: hand, spine (cervical or lumbar), knee, or hip. All were required to have symptomatic OA in at least one joint site. The control group comprised 712 subjects (409 women, 303 men; mean age 59 years, range 30–79) that were frequency-matched to the probands only for age ($\pm$ 5 years) and geographic region. They were recruited by random sampling of the population by telephone and not further screened for the presence or absence of OA. The London collection comprised 317 controls (mean age 53 years, range 36–71) and 159 OA (mean age 59 years, range 39–79) samples (all UK Caucasian women) selected randomly (a subject from each twin pair with independence of the OA status of the other twin) from the Twins UK registry of the Twin Research and Genetic Epidemiology Unit in London. Hand osteoarthritis was defined as $\geq$3 joints of both hands affected with osteoarthritis ($n = 72$). The definition for hip as for knee OA was a K/L grade of 2 or higher in one or both knees ($n = 71$) or one or both hips ($n = 71$; 5 of them also with OA in the knee). Patients and controls from each collection gave their informed consent. Each collection of samples has obtained the approval of the relevant ethics committee.

SNP prediction: The 18 ADAM and the 31 ADAMTS (except ADAMTS5[20]) proteases with available genome sequence were searched for non-synonymous SNPs (nsSNPs) in the dbSNP database. The 152 nsSNPs that were found were filtered for validation status. Likely functional consequences of the validated nsSNPs were assessed with two prediction softwares: SIFT (Sorting Intolerant From Tolerant)[31](available at http://blocks.fhcrc.org/sift/SIFT.html) that uses alignment to orthologous and homologue protein sequences, and PolyPhen (Polymorphism Phenotyping)[32] (available at http://genetics.bwh.harvard.edu/pph/index.html) that is based on empirical rules derived from phylogenetic and structural information. Four nsSNPs were predicted as likely deleterious: rs1054480 C/T in ADAMTS2, rs4747096 A/G in ADAMTS14, rs1019747 T/C in ADAMTS16, and rs3740199 G/C in ADAM12. We also searched PubMED for functional evidence of SNPs modifying ADAM or ADAMTS expression, but none was found.

nsSNP Genotyping: The three sample collections of the first set and the Corunna collection were genotyped by multiplex single-base extension in Santiago. PCR fragments were amplified in a multiplex PCR reaction (Qiagen Multiplex PCR, Valencia, CA, USA). Oligonucleotide sequences and PCR conditions are detailed in Supplementary Table I. Single-base extension reactions were done with the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA). The genotype call rate was 98.2 % for all nsSNPs across sample collections and across patients and controls. Several samples with different genotypes were sequenced to check for accuracy of results with the Big Dye Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The rs4747096 nsSNP was studied also in samples from the Leiden GARP and London (Twins UK) collections. The Leiden samples were genotyped by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions (call rate 94 % cases, 97.7 % controls). The London samples were genotyped (call rate 98.9 %) at Kbioscience Ltd (Hertfordshire UK) using the KASPar chemistry, which is a competitive allele-specific PCR genotyping system using fluorescence resonance energy transfer (FRET) quencher cassette oligonucleotides (http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm).

Statistical analysis: A customized version of the Statistica (Statsoft, Tulsa OK) software was used. Allele frequencies, odds ratios (O.R.) and their 95% confidence intervals (95% CI) were calculated. Comparison of allele frequencies was done using two by two contingency tables with chi-square tests. This statistic test was also used for carrier analysis, in which homozygotes for the minor allele and heterozygotes were pooled together as the carrier group and homozygotes for the major allele were the non-carrier group. Evidence of a gene dose effect was evaluated with univariate logistic regression applying an additive genetic model, in which the genotype of each subject is the independent variable with values linearly increasing with allele dose (codes were: 0 for AA, 1 for Aa and 2 for aa genotypes). For stratification analysis, female patients were compared with female controls and male patients with male controls. Data from populations of Santiago, Thessaly and Oxford were combined

using the Mantel-Haenszel test for the 2 × 2 contingency tables stratifying by collection of samples. This approach considers allele effect sizes in the form of odds ratios but it does not pool allele frequencies and is not affected by heterogeneity in these frequencies. However, it requires homogeneity of effect size (O.R.) across collections, which was assessed with the Breslow-Day test. Power and sample size calculations were done with the Power and Sample Size software for a value of $\alpha = 0.05$[33]. The Leiden collection required specific assays to compensate for familial relationships among the OA patients: strength of association was assessed with logistic regression analysis performed in STATA and robust standard errors were estimated from the variance between sib pairs.

Results

Search for SNPs affecting function of any of the ADAM or ADAMTS proteases (except ADAMTS5) led to selection of four nsSNPs likely to have deleterious effects. These four nsSNPs were studied in three sample collections that included 1412 THR, 781 TKR, 242 HOA and 1185 control samples from Santiago (Spain), Thessaly (Greece) and Oxford (United Kingdom). Genotyping was successful in most samples with results that were in Hardy-Weinberg equilibrium in each of the sample collections. Allele frequencies showed that the nsSNPs rs1054480 in ADAMTS2 and rs1019747 in ADAMTS16 were unrelated with any of the OA phenotypes analyzed, TKR, THR or HOA both in each of the individual collections (Table I) and in the combined analysis of the three collections (Table II). These results were not affected by gender stratification.

Minor allele frequency (MAF) of the rs3740199 nsSNP in ADAM12 was similar in cases and controls (Table I), but it showed significant MAF difference between THR men and control men from the Santiago collection (60.8 % vs. 52.2 %, respectively, $P = 0.04$) although this difference was not observed in men from the Oxford collection (53.8 % in THR vs. 53.6 % in controls) or in the combined analysis ($O.R._{M\text{-}H}$ (odds ratio. Mantel-Haenszel) = 1.11, 95 % C.I 0.9−1.3; Table II).

The rs4747096 A/G nsSNP in ADAMTS14 was significantly associated to OA in two groups of patients (Table I). The clearest difference was between patients with TKR and controls from the Oxford collection (MAF 21.6 % vs. 16.0 %, respectively) that was predominant in women (24.6 % vs. 15.7 % in cases and controls, respectively) with an O.R. of 1.75 (95 % C.I = 1.3−2.4). Men from Oxford showed not significant difference (18.0 % vs. 16.4 % in cases and controls, respectively). There was some indication of a dominant effect of the G allele in the Oxford's TKR women because the association was stronger in carrier analysis (O.R. = 2.05, 95% C.I. 1.4−3.0; $P = 0.0001$) than in allelic analysis (above) or an additive genotype model (O.R. = 1.78, 95% C.I. 1.3−2.4; $P = 0.0003$). TKR samples from the Santiago collection showed also a modest increase in the G allele frequency (Table I), but it was not significant. The Thessaly samples were neutral, or slightly in the same direction than the Oxford samples (O.R. for women = 1.10, 95% C.I. = 0.7−1.7). Pooled analysis of the three groups of TKR patients showed significant association that was specific of women (Table II; $O.R._{M\text{-}H}$ for women = 1.41, 95 % C.I = 1.1−1.8). TKR men showed a non-significant increase in G allele frequency.

The second group of patients that showed significant association to the ADAMTS14 nsSNP was the HOA group from Santiago, which was the unique available with this phenotype. In this instance, the G allele was also more frequent in patients than in controls (Table I; O.R. = 1.37, 95 % C.I = 1.0−1.9), but the increase was similar in women (O.R. = 1.43, 95 % C.I = 0.9−2.2) and men (O.R. = 1.47, 95 % C.I = 0.7−3.1). The minor allele of the ADAMTS14 nsSNP also showed a trend to higher frequencies in the two available groups of THR patients (Table I) and they remained as a non-significant trend ($P = 0.08$) in the combined analysis (Table II).

Given the interesting results obtained with the rs4747096 ADAMTS14 nsSNP we extended our analysis to other collections of OA samples (Table III). The Corunna samples were of symptomatic knee OA and were compared with the Santiago controls, as Santiago and Corunna share the same population and are only 60 Km apart. This group showed also a nominal increase in G allele frequency, but only among women (17.9 % vs. 15.2 % in controls) and this change was not significant (O.R. = 1.21, 95 % C.I. = 0.8−1.9). The samples from the GARP study have been selected for sib pair concordance in a generalized OA phenotype. They showed no change in the frequency of the rs4747096 alleles. Finally, we obtained rs4747096 genotypes from some samples with radiographic OA of the Twins UK registry. They did not show significant frequency changes (Table III), but this collection was the single one in which OA patients showed a nominal decrease in the MAF of the rs4747096 nsSNPs.

Discussion

This study has examined OA genetic association with four nsSNPs that likely are affecting genes of potential importance in OA, the ADAMTS and ADAM proteases.[8,16,23–25] It was not intended as a comprehensive assessment of these two protease families because genetic variation in these genes is much wider than the four nsSNPs studied here. Selection of putative deleterious nsSNPs increases the chances of finding association in a genetic study[34,35]. However, the most likely outcome is lack of association even in this subgroup of nsSNPs. In agreement with this expectation, we did not find association with three of the four nsSNPs studied, in ADAMTS2, ADAMTS16 and ADAM12, respectively. Only the ADAM12 rs3710199 nsSNP deserves a specific commentary because this nsSNP has previously been studied in relation with knee OA susceptibility. The minor allele was associated with osteophytes and change in osteophyte grade in a prospective study of UK women with radiographic knee OA[25]. A subsequent study in UK patients with symptomatic knee OA showed no association with this nsSNP, although a haplotype of the same gene was associated[36]. Our results showed lack of association of this nsSNP with knee OA (ascertained as TKR) and indicated that it is not associated to THR or HOA. However, it should be noted that osteophyte formation and other polymorphisms in the ADAM12 gene were not investigated in our study.

In contrast, the rs4747096 nsSNP in ADAMTS14 showed significant association with some OA phenotypes, but not with others. Association was clear in the TKR group, and especially in women from the UK. In addition, association was present in clinical hand OA, HOA. The strength of association was similar in HOA and in TKR females as evaluated by the O.R. (1.37 vs. 1.41, respectively). However, the limited number of available HOA samples made this result more uncertain than the obtained in TKR females. We also noted a trend to association with the THR group. In this case, the effect size was lower (O.R. = 1.14), but the large sample size of our THR group (1412 patients) allowed for better detection sensitivity. These are promising but not definitive results. The association to TKR and HOA will require additional studies for confirmation. Also additional studies will be needed to delineate the effects that this

Table I

Minor allele frequencies of four putative damaging nsSNPs, rs1054480 in ADAMTS2, rs1019747 in ADAMTS16, rs3740199 in ADAM12 and rs4747096 in ADAMTS14, in patients with three OA phenotypes, requiring joint replacement of the knee, TKR, or the hip, THR, or with hand OA, HOA, compared with controls without OA from three sample collections, Santiago, Thessaly and Oxford. Minor allele percentages of the total group are followed by the separated women/men results

Collection	Group[a]	ADAMTS2			ADAMTS16			ADAM12			ADAMTS14		
		T %	P	O.R. (95% C.I.)	C %	P	O.R. (95% C.I.)	C %	P	O.R. (95% C.I.)	G %	P	O.R. (95% C.I.)
Santiago	Control (n = 588, 230/358)[a]	29.7 31.3/28.8			36.0 34.8/36.9			55.6 60.9/52.2			16.2 15.2/16.8		
	TKR (n = 524, 422/102)	31.3 32.2/27.4	0.6	1.07 (0.8−1.4)	36.8 36.4/38.2	0.8	1.03 (0.8−1.3)	55.3 56.3/50.9	0.9	0.98 (0.8−1.2)	17.4 16.6/20.6	0.6	1.09 (0.8-1.5)
	THR (n = 614, 370/242)[b]	26.5 25.0/29.2	0.2	0.85 (0.7−1.1)	36.3 37.4/35.0	0.9	1.01 (0.8−1.3)	58.8 57.6/60.8	0.3	1.14 (0.9−1.4)	18.4 18.8/17.9	0.3	1.17 (0.9-1.6)
	HOA (n = 484, 426/54)[b]	28.1 28.9/24.1	0.6	0.92 (0.7−1.2)	33.5 33.9/29.6	0.4	0.89 (0.7−1.15)	57.3 57.8/51.8	0.6	1.06 (0.8−1.4)	20.9 20.4/23.1	0.047	1.37 (1.0-1.9)
Thessaly	Control (n = 386, 274/112)	31.9 29.5/37.5			33.3 33.8/32.1			62.0 62.6/60.7			11.5 11.1/12.5		
	TKR (n = 318, 278/40)	35.9 35.4/40.0	0.3	1.19 (0.9−1.6)	29.6 28.8/35.0	0.3	0.84 (0.6−1.2)	56.7 58.4/45.0	0.2	0.80 (0.6−1.1)	11.4 12.1/7.5	1.0	0.99 (0.6-1.6)
Oxford	Control (n = 1396, 712/684)	27.8 26.1/29.6			38.5 39.3/37.8			53.3 53.1−53.6			16.0 15.7/16.4		
	TKR (n = 720, 392/328)	28.2 27.0/29.5	0.9	1.02 (0.8−1.3)	36.1 37.8/34.3	0.4	0.90 (0.7−1.1)	51.4 52.4−50.3	0.4	0.93 (0.8−1.1)	21.6 24.6/18.0	0.001	1.44 (1.1-1.8)
	THR (n = 2210, 1258/952)	28.8 28.0/29.8	0.5	1.05 (0.9−1.2)	41.2 40.3/42.4	0.1	1.12 (0.97−1.3)	54.7 55.5−53.8	0.4	1.06 (0.9−1.2)	17.8 16.9/19.0	0.2	1.13 (0.9−1.4)

[a]n = total number of alleles, number of alleles from women/from men.

[b]A total of three samples lacked sex information.

Table II

Pooled analyses of the allele effect sizes (O.R.) corresponding to the three sample collections and the four putative damaging ADAMTS and ADAM nsSNPs of Table I in the patient groups with TKR and THR. Analyses were done with the Mantel-Haenszel approach and including sex stratification

Group[a]	ADAMTS2			ADAMTS16			ADAM12			ADAMTS14		
	$O.R._{M\text{-}H}$[b] (95 % C.I.)	P	$P_{B\text{-}D}$[c]	$O.R._{M\text{-}H}$ (95 % C.I.)	P	$P_{B\text{-}D}$	$O.R._{M\text{-}H}$ (95 % C.I.)	P	$P_{B\text{-}D}$	$O.R._{M\text{-}H}$ (95 % C.I.)	P	$P_{B\text{-}D}$
TKR (n = 1562) (n ctrl = 2370)	1.06 (0.9−1.2)	0.4	0.7	0.9 (0.8−1.1)	0.4	0.5	0.92 (0.81−1.05)	0.2	0.6	1.25 (1.05−1.5)	0.009	0.2
TKR women (n = 1092) (n ctrl = 1216)	1.10 (0.9−1.3)	0.3	0.5	0.9 (0.8−1.1)	0.5	0.4	0.90 (0.75−1.06)	0.2	0.7	1.41 (1.1−1.8)	0.002	0.1
TKR men (n = 470) (n ctrl = 1154)	0.99 (0.8−1.3)	1.0	0.9	0.9 (0.7−1.2)	0.6	0.7	0.86 (0.69−1.07)	0.2	0.4	1.12 (0.8−1.45)	0.5	0.5
THR (n = 2824)[d] (n ctrl = 2370)	1.00 (0.9−1.1)	0.9	0.3	1.08 (0.96−1.2)	0.2	0.4	1.08 (0.96−1.21)	0.2	0.6	1.14 (0.98−1.3)	0.08	0.8
THR women (n = 1628) (n ctrl = 1216)	1.01 (0.8−1.2)	0.9	0.1	1.05 (0.9−1.2)	0.5	0.8	1.04 (0.89−1.23)	0.6	0.2	1.14 (0.9−1.4)	0.2	0.5
THR men (n = 1194) (n ctrl = 1154)	1.01 (0.8−1.2)	0.9	1.0	1.13 (0.95−1.3)	0.2	0.2	1.11 (0.94−1.32)	0.2	0.1	1.16 (0.9−1.5)	0.2	0.7

[a]n = total number of alleles of Santiago, Thessalay and Oxford.

[b]Mantel-Haenszel odds ratio.

[c]Breslow-Day heterogeneity test.

[d]One sample (two alleles) haven't got defined sex.

ADAMTS14 nsSNP could have in other OA forms including the THR group.

We already tried to obtain more information by analyzing the ADAMTS14 nsSNP in other three sample collections. Unfortunately, the results were inconclusive. The symptomatic KOA collection from Corunna showed a trend to increased frequency of the G allele in the women's group, consistent with the observed in TKR women. No change was observed in the generalized OA patients from the GARP study or in the radiographic OA samples from the Twins UK registry. These three collections were of different OA phenotypes and each of them provided limited statistical power. These two aspects make the results in these three collections of uncertain interpretation. Available evidence shows that each OA phenotype is dependent on different genetic factors or with different strength[1–3]. In addition, effects of the ADAMTS14 nsSNP seem small and their demonstration requires large studies. Sample sizes large enough to detect an effect with O.R. = 1.3 and 80% power were only available in the pooled analyses of TKR and of THR patients because over 700 cases and 700 controls are needed. The Corunna, Leiden and London collections have only 39 %, 62 %, and 33 % power, respectively, for this same effect.

Confidence on the association between symptomatic knee OA in women and ADAMTS14 genetic variation has been reinforced by results of a WGAS done in the UK and communicated in a recent meeting[37]. This study has analyzed more than 400,000 SNPs spread over the genome in women knee OA patients and the SNPs showing the largest differences were genotyped in additional collections of samples totalling more than 3,000 women. One of the few SNPs that remained associated after replication was a SNP in the forth intron of ADAMTS14 that showed a pooled $O.R._{M\text{-}H} = 1.2$ (95 % C.I. = 1.02–1.41). This study did not overlap with the samples included in our TKR collections. Therefore, two independent studies with a similar OA phenotype and large sample sizes have found association with SNPs in the ADAMTS14 gene. However, these concordant results do not amount to replication because the SNPs are different and not in linkage disequilibrium and confirmation in new studies is still required.

Further analyses will be also necessary to show the effects of the E1049G change in ADAMTS14 function and to explore other ADAMTS14 polymorphims. Study of this gene will not be easy because its 22 exons are spread over 89.6 Kb in chromosome 10q22.1 and because little is still known about ADAMTS14 function[38–40]. The rs4747096 nsSNP determines a change of glutamic acid for glycine in a segment of the protein of unclear functional significance. It was predicted as damaging by PoylPhen, a software application with specificity over 90 %[41]. It provides a global evaluation but does not indicate the likely damaging effect. A clue of the possible functional relevance was obtained by comparative phylogenetic analysis. A glutamic acid in this position is exclusive of the human ADAMTS14 gene. Glycine is the ancestral allele as this is the amino acid in ADAMTS14 orthologues in chimpanzee and rhesus macaque. However, the glutamic acid allele is more common than the glycine allele in Europeans, Africans and Asians, raising the possibility of some advantage associated to it that will have favored its positive selection in humans. However, little else can be said until more is known on ADAMTS14 function. Most available information has been inferred by its similarity to two other ADAMTS, ADAMTS-2 (56 %) and -3 (63 %)[40]. The three are able to cleave the N-telopeptides of procollagen, a process that takes place in collagen monomers before they are incorporated into fibers. Expression of the three propeptidases has been found to be up-regulated in OA cartilage[23,24]. This over-expression can be related with cartilage repair and production of matrix components as part of the anabolic compensation to cartilage degradation. ADAMTS14 is produced in a latent form and, after activation, it processes procollagen I in an *in vitro* system. However, no other substrates have been examined and its natural *in vivo* substrate is unknown[40]. The relevance of ADAMTS14 activity on procollagen I processing is unclear as it is not able to replace ADAMTS2 deficiency in Ehlers-Danlos syndrome type VIIC[42].

In conclusion, we have found that a change of glutamic acid for glycine in ADAMTS14 was associated with women knee OA requiring joint replacement and with clinical hand OA. The first association was more robust because of the larger sample size and because of evidence from other study of association between ADAMTS14 genetic variation and symptomatic knee OA in women. Our results suggest also that the same nsSNP could have association, but weaker, with hip OA requiring prosthesis. These results require confirmation in well powered studies. Also,

Table III

Minor allele frequencies of the ADAMTS14 nsSNPs rs4747096 in three sample collections that are different from the analyzed in Tables I and II. These collections included patients with varied OA phenotypes: symptomatic knee OA in the Corunna collection, familial OA at multiple sites in the Leiden collection and women with radiographic OA in the London collection. Minor allele percentages of the total group are followed by the separated women/men results

Collection	Group[a]	ADAMTS14		
		G %	*P*	O.R. (95% C.I.)
Corunna	Controls[b] (n = 588, 230/358)	16.2 15.2/16.8		
	KOA (n = 530, 444/86)	16.7 17.9/10.7	0.8	1.04 (0.75–1.4)
Leiden	Controls (n = 1424, 818/606)	16.9 16.4/17.7		
	GARP (n = 716, 584/132)	17.5 16.6/21.2	0.8	1.04 (0.8–1.3)
London[c]	Controls (n = 634)	18.6		
	ROA (n = 318)	15.4	0.2	0.79 (0.55–1.15)

[a]n = Number of alleles, number of alleles from women/from men; KOA = sympthomatic and radiographically confirmed knee OA; GARP = Genetics, Arthrosis and Progression, sib pairs concordant for OA at multiple sites; ROA = radiographic OA of the hand, knee or hip.

[b]Controls from the Santiago collection.

[c]All samples of this collection are from women.

a systematic study of ADAMTS14 variation should be done to uncover possible additional polymorphisms. Other OA phenotypes we have examined showed lack of association. The incomplete knowledge of ADAMTS14 functions hampers prediction of its possible role in OA, but it is likely that deficient ADAMTS14 could lead to incompletely processed collagen that will cause detrimental effects on cartilage matrix structure and function.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

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