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Missense single nucleotide polymorphism of the ADAM12 gene is associated with radiographic knee osteoarthritis in middle-aged Estonian cohort

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Summary

Objective: One of the recognized candidate genes of osteoarthritis (OA) is the ADAM metallopeptidase domain 12 (meltrin alpha) gene. We investigated the potential role of two single nucleotide polymorphisms (SNP) of the ADAM12 gene in susceptibility to radiographic knee OA and its progression in an Estonian cohort.

Methods: The rs3740199 and rs1871054 polymorphisms were genotyped according to restriction fragment polymorphism in a population-based cohort consisting of 189 subjects selected from the age group 32–55 years. The radiological features of OA were measured in the tibio- and patellofemoral joints (PFJ). The X-ray investigation was repeated 3 years later for estimation of OA progression.

Results: We found statistically significant association between rs3740199 polymorphism and patellofemoral OA in male patients ($P = 0.014$), genetic risk was mostly related to CC homozygosity. The same SNP also affected the presence of advanced grade (II + III) osteophytes in the whole group ($P = 0.042$) and the occurrence of osteophytes on the patellar margins in the PFJ ($P = 0.046$). In OA progression the most significant association was found between joint space narrowing of the tibiofemoral joint and rs3740199 SNP in women ($P = 0.018$). The rs1871054 polymorphism was not related to OA susceptibility or to progression traits. In our study the haplotype GC (rs3740199/rs1871054) was associated with reduced risk for development of osteophytes in the PFJ ($P = 0.041$).

Conclusions: We conclude that rs3740199 polymorphism may affect occurrence of knee OA and its progression. We also hypothesize that the genetic contribution of ADAM12 to OA is remarkably gender-dependent and anatomical site-specific.

Key words: Knee osteoarthritis, ADAM12 gene, SNP, rs3740199, Patellofemoral joint.

Introduction

Osteoarthritis (OA) is the most common arthritic disorder and a leading cause of musculoskeletal disability in developed countries[1]. In addition to age, sex, body weight and trauma, the genetic background also contributes to the risk of OA. A classical twin study estimated the heritability of knee and hip OA to be 39–65% in affected women[2]. The genetic background seems to be less important in men[3].

In OA the knee is the most significant clinically affected site[4]. Owing the tri-compartmental anatomical structure of the knee joint (medial and lateral tibiofemoral joint (TFJ), and patellofemoral joint (PFJ)), numerous radiographic patterns of knee OA are possible. Traditionally, knee OA has been regarded as a disorder of the TFJ. Recently, increasingly more attention has been given to the importance of patellofemoral OA as an important subgroup of knee OA[5]. In the opinion of some authors patellofemoral OA should be considered a distinct entity[6]. Epidemiological studies suggest that the risk factors associated with the disease are different for patellofemoral OA and tibiofemoral OA[7,8]. At present it is authentically unknown if the genetic risk factors contributing to development of OA in TFJ and PFJ are different as well. However, a recent thorough British study investigating the risk of tibio- and patellofemoral OA in siblings reported a relative risk of 2.9 (95 % CI 2.3–3.7) for tibiofemoral OA and 1.7 (95 %CI 1.4–2.2) for patellofemoral OA after adjusting for environmental factors[9].

In fact, since 2002 the genetic pattern of OA has been investigated by genome-wide linkage analysis and numerous association studies of candidate genes have been performed with a total number of more than 50 genes[10]. The most convincing evidence for involvement in OA has been noted for the interleukin 1 (IL1) gene cluster, the matrilin 3 gene (MATN3), the IL-4 receptor α chain gene, the secreted frizzled related protein 3 gene (FRZB), the asporine gene and the metalloproteinase gene ADAM12[11]. The association between OA and the ADAM12 gene has probably been the least studied[12,13]. This gene encodes a member of the disintegrin and metalloprotease protein family. The members of this family are implicated in a variety of

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biological processes involving cell–cell and cell–matrix interactions in fertilization, muscle development and neurogenesis[14].

The aim of the present pilot study was to investigate the potential role of two single nucleotide polymorphisms (SNPs), the rs3740199 and rs1871054, of the ADAM12 gene in susceptibility to radiological knee OA and its progression in an Estonian cohort aged 32–55 years.

Patients and methods

SUBJECTS

The primary survey was conducted in a small South-Estonian town of Elva in 2002. A questionnaire on knee problems was sent to all 559 subjects aged 32–55 years (family doctor's list) and a total of 348 responses were obtained. Of all contacted subjects 190 (118 women, 82 men) participated in an in-depth examination (X-ray and ultrasonography of both knee joints, functional knee tests). The radiograph of one participant was considered unsuitable and he was excluded from final analysis. Blood samples were collected for assessment of bone and cartilage turnover markers[15] and all subjects were subjected to genetical investigation.

Standardised weight-bearing antero-posterior radiographs of TFJ and axial radiographs of the knee joint with PFJ in 60° flexion were used for radiographic assessment of knee OA[16]. The two main features of knee OA, presence of joint space narrowing (JSN) and osteophyte development, were estimated independently by two radiologists in both TFJ and PFJ according to the grading system (grades 0–III) of Nagaosa *et al.*[17]. The highest grade was regarded as the stage of OA in the corresponding joint. The highest grade of OA in TFJ or PFJ was regarded as the global stage of knee OA and the highest grade of osteophytes in TFJ or PFJ was regarded as the global grade of osteophytes.

To estimate the progression of knee OA, a similar follow-up study was performed 3 years later with a total number of 141 participants (98 women). Progression was defined as a change in the stage of JSN and grade of osteophytes with or without a change in the global grade of knee OA.

The radiographs of the participants from the primary study who showed a reduction from higher radiographic knee OA grade at baseline to lower grade at follow-up ($N = 10$) were reassessed. The final decision about the knee OA grade at baseline and at follow-up was made by consensus between the same radiologists.

The study was approved by the Ethics Committee of the University of Tartu and informed consent was obtained from all subjects.

GENOTYPING

Genomic DNA was purified from the samples of peripheral venous blood by salt extraction.

The polymorphisms of the ADAM12 gene (rs3740199 and rs1871054) were amplified by polymerase chain reaction (PCR) and analyzed by restriction fragment polymorphism (RFLP). The forward primer (5′ GCT GAT GAA GTT GTC AGA GCC T 3′) and the reverse primer (5′ GGA TCC CTC ATC AGC ACT GTC AC 3′) were used for amplification of rs3740199 (48 C > G). The size of the fragments of the PCR products after restriction with PpiI (Fermentas) was 231 bp for theC-allele (Arginine) and 265 bp for the G-allele (Glycine).

The rs1871054 (C > T) were amplified with the forward primer (5′ TCT GCT TTG ACA GTG TGC ATG GCT 3′) and the reverse primer (5′ GCT CTC CAG AGT AGC ACA GGT CAC 3′). Eco 91I (Fermentas) was used for RFLP and the final product size was 131 bp for the C allele and 150 bp for the T allele.

The PCR program consisted of the following steps. The first step was initial denaturation in which the reaction was incubated for 4 min at 95°C. In the second step, DNA was amplified for 37 cycles of denaturation at 95°C for 1 min, annealing at 60°C (rs3740199) and at 62°C (rs1871054) for 3 min, and extension at 72°C for 60 s.

STATISTICAL ANALYSIS

The association between the SNPs and different radiographic features of knee OA was evaluated by the chi-square test and by logistic or linear regression models in the *R* environment ver. 2.4.0 (The R Foundation for Statistical Computing, Boston, MA)[18]. The haplo.stats (haplo.glm) package in the R-program was used to estimate the haplotypes and the associations of the investigated SNPs. The Hardy-Weinberg equilibrium (HWE) was evaluated by the genetics package (HWE.test). The age and the body mass index (BMI) of the subjects were used as the covariates in most statistical calculations.

Result

RADIOLOGICAL KNEE OA GRADES AND ALLELIC DISTRIBUTION OF SNPs

On examination, 118 (87 women and 31 men) of the189 participants were diagnosed with different stages (I–III) of knee OA. Seventy-one subjects (52 women and 19 men) without radiological features of knee OA were classified as the control group. The subjects with stage I knee OA formed the largest group (49% of all participants). Advanced radiological features of knee OA (grades II + III) were found in 25 patients. Twenty-one of the 118 patients with features of radiological knee OA reported absence of knee pain or other knee problems.

The frequencies (%) of SNPs genotypes for the study groups are presented in Table I. All genotypes of both SNPs were found to be in HWE.

The calculated allelic frequency in rs3740199 SNP was 30% for the G-allele and 70% for the C allele. For s1871054 polymorphism the following distribution was obtained: 49.5% and 50.5% for the C and T alleles, respectively. The estimated distribution of SNP's genotypes according to two main features of OA is shown in Table II. None of the estimated haplotypes (rs3740199/rs1871054) had a frequency lower than 5% (Table III).

CONSEQUENCE OF GENETIC ASSOCIATION FOR RADIOGRAPHIC KNEE OA SUSCEPTIBILITY TRAITS

In order to investigate possible association between the genotyped ADAM12 SNPs and radiological knee OA, the rs3740199 and rs1871054 alleles were collated to (1) global grade of knee OA and global grade of osteophytes, (2) grade of OA and two main features of OA (osteophytes, JSN) in TFJ and PFJ.

We found significant association between rs3740199 polymorphism and patellofemoral OA in male patients (OR 3.55, $P = 0.014$)(Table IV), while CC homozygotes showed the highest risk compared with the other genotypes. No such association was found in the group of women. A detailed investigation of osteophytes in both joints revealed that rs3740199 is linked to the presence of advanced grade (II + III) osteophytes in the whole group ($P = 0.042$). Comparison of the occurrence of osteophytes separately at different sites of TFJ and PFJ revealed association between rs3740199 and presence of osteophytes in PFJ, particularly on the lateral patellar margins (surface) in affected subjects ($P = 0.046$).

We did not find statistically significant association between rs3740199 SNP and the other investigated radiological parameters.

No association was found between any of the tested parameters and rs1871054 SNP.

Haplotype analysis

We found that the GC haplotype was associated with decreased risk of radiographical OA in PFJ in men (Table IV). The same haplotype was also associated with reduced risk for development of osteophytes in PFJ in the whole group (OR = 0.24, $P = 0.041$). Most likely, the trend of the protective effect of the haplotype regarding osteophytes was present in women ($P = 0.058$). No association was observed between the other haplotypes and any traits of knee OA.

Table I
Study groups and distribution of ADAM12 gene genotypes in subjects with and without radiographical knee OA in Estonian cohort at baseline (2002)

ADAM12 gene	Tibiofemoral knee OA (TFOA)				Patellofemoral knee OA (PFOA)			
	Subjects without	Subjects with	OR (95% CI)	*P*-value	Subjects without	Subjects with	OR (95% CI)	*P*-value
rs3740199 genotypes								
CC								
Whole group	65 (53%)	28 (42%)	0.85 (0.29–2.49)	0.76	41 (44%)	53 (55%)	1.01 (0.37–2.80)	0.97
Male	16 (46%)	6 (40%)	0.71 (0.1–4.89)	0.72	7 (28%)	16 (64%)	10.7 (1.05–109.8)	**0.045**
Female	49 (56%)	22 (43%)	0.9 (0.24–3.30)	0.82	34 (51%)	37 (51%)	0.36 (0.09–1.45)	0.15
CG								
Whole group	46 (37%)	32 (49%)	1.39 (0.47–4.09)	0.55	43 (47%)	34 (35%)	0.65 (0.23–1.83)	0.42
Male	15 (43%)	7 (47%)	0.93 (0.14–6.37)	0.94	13 (52%)	8 (32%)	3.46 (0.34–34.8)	0.29
Female	31 (35%)	25 (49%)	1.61 (0.43–5.98)	0.48	30 (45%)	26 (36%)	0.29 (0.07–1.18)	0.08
GG								
Whole group	12 (10%)	6 (9%)	1*		8 (9%)	10 (10%)	1*	
Male	4 (11%)	2 (13%)	1*		5 (20%)	1 (4%)	1*	
Female	8 (9%)	4 (27%)	1*		3 (4%)	9 (13%)	1*	
G-allele %†								
Whole group	28.5	33.3	0.78 (0.23–1.94)	0.37	32.1	27.8	0.86 (0.55–1.33)	0.50
Male	32.9	36.6	1.18 (0.48–2.89)	0.71	46	20	0.33 (0.14–0.79)	**0.013**
Female	26.7	32.4	1.28 (0.75–2.17)	0.37	26.9	30.5	1.24 (0.74–2.08)	0.42
rs1871054 genotypes								
TT								
Whole group	30 (24%)	18 (27%)	1.24 (0.53–2.9)	0.61	24 (26%)	24 (25%)	0.7 (0.31–1.59)	0.39
Male	10 (28%)	4 (27%)	1.07 (0.18–6.21)	0.94	7 (28%)	7 (28%)	0.57 (0.11–2.87)	0.49
Female	20 (23%)	14 (27%)	1.34 (0.51–3.56)	0.55	17 (25%)	17 (23%)	0.75 (0.29–1.94)	0.55
CT								
Whole group	62 (51%)	33 (50%)	1.08 (0.51–2.28)	0.84	49 (54%)	46 (47%)	0.66 (0.32–1.35)	0.25
Male	17 (49%)	8 (53%)	1.19 (0.25–5.68)	0.83	14 (56%)	11 (44%)	0.45 (0.10–1.93)	0.28
Female	45 (51%)	25 (49%)	1.06 (0.45–2.50)	0.88	35 (52%)	35 (49%)	0.75 (0.33–1.70)	0.49
CC								
Whole group	31 (25%)	15 (23%)	1*		19 (20%)	27 (28%)	1*	
Male	8 (23%)	3 (20%)	1*		4 (16%)	7 (28%)	1*	
Female	23 (26%)	12 (24%)	1*		15 (23%)	20 (28%)	1*	
C allele %†								
Whole group	50.4	47.7	0.9 (0.59–1.37)	0.62	47.2	51.5	1.19 (0.79–1.78)	0.41
Male	47.1	46.6	0.98 (0.42–2.31)	0.97	44	50	1.27 (0.58–2.80)	0.55
Female	51.7	48	0.86 (0.53–1.41)	0.56	48.5	52.1	1.15 (0.72–1.85)	0.55

*Reference.
†Minor allele.

Three years follow-up of progression of knee OA features

The prevalence of radiographic knee OA in the study cohort was 62% at baseline and 86% after 3 years of follow-up.

The most significant association was found between rs3740199 and progression of OA assessed on the basis of JSN of TFJ in women (OR 2.66, 95% CI 1.19–5.98, $P = 0.018$). The observed summary progression (osteophytes + JSN in TFJ) of tibiofemoral OA in female patients was also associated with the same SNP (OR 1.96, 95% CI 1.00–3.84, $P = 0.048$). However, after adjusting for age and BMI, the association lost statistical significance ($P = 0.064$). No significant association was observed between rs3740199 SNP and progression of OA in men.

The rs1871054 SNP was not related to any traits of OA progression.

Discussion

In the present study we evaluated the association between ADAM12, a recently discovered OA candidate gene, and presence and progression traits of knee OA in a younger to middle-aged general population (32–55 years). Two SNPs of the gene were investigated. The first, rs3740199 (G > C) in the second exon, represents missense polymorphism leading to substitution of Gly48Arg. The rs1871054 (C > T) is located in the eleventh intron. The distribution of the alleles of rs1871054 SNP (49.5% C and 50.5% T) in the Estonian cohort was similar to that in the HapMap European population[19]. The number of the ancestral G-allele carriers of rs3740199 SNP, on the contrary, was significantly lower in the studied population compared to the Caucasians reported by HapMap (30% vs 61%). However, our finding is similar to that found in a recently published British Chingford Study where the G-allele of rs3740199 was observed as a minor allele[12].

Preliminary data about the possible role of the ADAM12 gene in knee OA was recently published by Valdes *et al.* (2004). The ADAM12 was selected alongside with other genes of interest, expressed differently in the healthy and in OA cartilage and synovium, as the potential affector gene implicated in knee OA pathogenesis[12]. This investigation, based on a Chingford Study cohort, UK (women aged

Table II
Distribution of ADAM12 gene genotypes in subjects with and without knee OA radiological features in Estonian cohort at baseline (2002)

ADAM12 gene	Osteophytes (OPH)				JSN			
	Subjects without	Subjects with	OR (95% CI)	*P*-value	Subjects without	Subjects with	OR (95% CI)	*P*-value
rs3740199 genotypes								
CC								
Whole group	62 (50%)	31 (48%)	1.00 (0.34–2.92)	1.00	48 (49%)	45 (50%)	0.94 (0.34–2.57)	0.90
Male	14 (42%)	8 (47%)	1.14 (0.17–7.69)	0.89	10 (37%)	12 (52%)	6.00 (0.60–60.2)	0.13
Female	48 (58%)	23 (48%)	0.96 (0.26–3.51)	0.94	38 (53%)	33 (48%)	0.43 (0.12–1.57)	0.20
CG								
Whole group	50 (40%)	28 (43%)	1.12 (0.38–3.31)	0.83	41 (43%)	37 (40%)	0.90 (0.32–2.52)	0.84
Male	15 (46%)	7 (41%)	0.93 (0.14–6.37)	0.94	12 (44%)	10 (44%)	4.17 (0.42–41.8)	0.22
Female	35 (38%)	21 (44%)	1.20 (0.32–4.48)	0.77	29 (41%)	27 (40%)	0.47 (0.13–1.72)	0.25
GG								
Whole group	12 (10%)	6 (9%)	1*		9 (9%)	9 (10%)	1*	
Male	4 (12%)	2 (12%)	1*		5 (19%)	1 (4%)	1*	
Female	8 (9%)	4 (8%)	1*		4 (6%)	8 (12%)	1*	
G-allele %†								
Whole group	29.8	30.8	1.03 (0.65–1.62)	0.92	30.1	30.2	1.03 (0.67–1.60)	0.89
Male	34.8	32.4	0.89 (0.37–2.15)	0.80	40.7	26.1	0.51 (0.22–1.20)	0.13
Female	28	30.2	1.08 (0.63–1.86)	0.78	26.1	31.6	1.36 (0.81–2.28)	0.25
rs1871054 genotypes								
TT								
Whole group	28 (23%)	20 (31%)	3.43 (0.64–3.43)	0.36	23 (24%)	25 (27%)	1.19 (0.58–2.66)	0.68
Male	9 (27%)	5 (29%)	1.48 (0.27–8.27)	0.66	8 (29%)	6 (26%)	0.63 (1.13–3.07)	0.56
Female	19 (21%)	15 (31%)	1.51 (0.57–4.00)	0.40	15 (21%)	19 (28%)	1.50 (0.55–3.83)	0.39
CT								
Whole group	65 (52%)	30 (46%)	0.95 (0.45–2.03)	0.89	51 (52%)	44 (49%)	0.94 (0.46–1.91)	0.87
Male	16 (49%)	9 (53%)	1.50 (0.32–7.12)	0.61	14 (52%)	11 (48%)	0.65 (0.16-2.72)	0.56
Female	49 (54%)	21 (44%)	0.82 (0.35–1.95)	0.66	37 (52%)	33 (48%)	1.06 (0.47–2.39)	0.89
CC								
Whole group	31 (25%)	15 (23%)	1*		24 (24%)	22 (24%)	1*	
Male	8 (24%)	3 (18%)	1*		5 (19%)	6 (26%)	1*	
Female	23 (25%)	12 (25%)	1*		19 (27%)	16 (24%)	1*	
C allele†%								
Whole group	51.2	46.2	0.82 (0.53–1.25)	0.35	50.5	48.3	0.92 (0.61–1.37)	0.67
Male	48.5	44.1	0.84 (0.37–1.93)	0.68	44.4	50	1.25 (0.57–2.75)	0.58
Female	52.2	46.9	0.81 (0.49–1.33)	0.40	52.8	47.8	0.82 (0.51–1.31)	0.40

*Reference.
†Minor allele.

47–63, $N = 749$), found some association between rs3740199 SNP and radiographic knee OA (score >2 by Kellgren/Lawrence, K/L) or presence of osteophytes in TFJ. In the present study, we failed to detect association between the distribution of investigated ADAM12 genotypes and radiological OA in TFJ (assessed by the radiographic Atlas of Knee OA[16]) in whole population or separately in men and women. We believe that the absence of association in our study can be explained by gender and age-related differences between the investigated cohorts. The study group consisted of female and male subjects, and the age of the participants (strongly restricted to the range 32–55 years) was younger compared with the Chingford Study. In addition, despite the fact that the K/L score >2 is comparable to the OA score >1 used in our study, some discrepancies could have resulted from the methodology of assessment of OA.

The PFJ compartment is frequently afflicted by the disease process and PFJ OA is associated with considerable pain and disability[4]. The patella demonstrates more severe degeneration earlier in the disease process compared with the juxtaposed femoral groove[20]. The present study is the first, to the best of our knowledge, to investigate the potential relationship between the ADAM12 gene and presence of patellofemoral OA. In this study rs3740199 SNP was associated with susceptibility to patellofemoral OA in male patients and the CC genotype of rs3740199 carried the highest risk in comparison with the other genotypes. The reliability and importance of this finding should be validated in further research.

Evaluation of the genetic background of not only the total score of OA but also of different processes and locations of OA was one of main tasks of our study. Analysis of the features of regenerative response of the cartilage through development of osteophytes revealed relationship between presence of advanced stage osteophytes in both joints (TFJ + PFJ) and rs3740199. In the cohort of the above mentioned Chingford Study rs3740199 SNP was significantly associated with presence of osteophytes[12]. Despite the circumstance that in our study the association of the gene was only revealed in the presence of the advanced stages of osteophytes (II and III), we believe that this finding is in concert with the data described first by Valdes *et al.*[12]. Analysis of different locations of OA related changes in PFJ demonstrated that rs3740199 polymorphisms are mainly associated with bone spurs on the lateral patellar margins.

Table III
The rs3740199/rs1871054 haplotype frequencies and associations with radiological OA in investigated population

Haplotype of rs3740199/ rs1871054	Frequencies %		OR (95% CI)
	Subjects without radiological traits of knee OA	Subjects with radiological traits of knee OA	
CC			
Whole group	33.9	37.5	1*
Male	28.3	40.3	1*
Female	35.2	36.4	1*
CT			
Whole group	37.2	31.5	0.42 (0.74–4.28)
Male	32.3	29.1	0.53 (0.13–2.08)
Female	40.3	32.8	0.77 (0.41–1.48)
GC			
Whole group	16.1	11.6	0.59 (0.24–1.42)
Male	16.4	8.1	0.23 (0.03–1.86)
Female	15.8	13	0.68 (0.25–1.79)
GT			
Whole group	12.8	19.4	1.43 (0.70–2.92)
Male	23	22,5	0.68 (0.21–2.14)
Female	8.7	17.8	2.34 (0.83–6.65)

*Reference.

No significant influence of rs3740199 was seen on the degradation of the cartilage tissue measured as JSN in both joints.

Another SNP investigated by us, rs1871054, showed no association with any OA susceptibility traits in the Estonian population. This finding differs from the data of Valdes and co-workers[21] who included the rs1871054 in the mathematical model of the additive effect of individual genes in predicting the risk of knee OA. However, further studies on larger material are needed to validate the association between rs1871054 and knee OA development.

Table IV
The rs3740199 SNP and rs3740199/rs1871054 (GC) haplotype associations with radiographic knee OA susceptibility features

	OR (95% CI)	*P*-value
rs3740199, investigated trait		
Presence of PFOA*		
Whole group	1.25 (0.80–1.94)	0.328
Male patients	**3.55 (1.29–9.76)**	**0.014**
Female patients	0.89 (0.53–1.52)	0.679
Presence of OPH (advanced stage II + III)*		
Whole group	**19.7 (1.12–345.00)**	**0.042**
Male patients†	–	–
Female patients	22.91 (0.79–663.98)	0.068
Presence of POPH*		
Whole group	**2.77 (1.02–7.53)**	**0.046**
Male patients	7.04 (0.82–60.71)	0.076
Female patients	1.92 (0.59–6.23)	0.277
rs3740199/rs1871054 GC, investigated trait		
Presence of PFOPH		
Whole group	**0.24 (0.06–0.94)**	**0.041**
Male patients	0.30 (0.02–4.10)	0.368
Female patients	0.20 (0.04–1.06)	0.058
Presence of PFOA		
Whole group	0.55 (0.24–1.32)	0.183
Male patients	**0.10 (0.01–0.95)**	**0.046**
Female patients	0.77 (0.30–1.99)	0.592

PFOA - OA of patellofemoral joint, OPH - osteophytes, POPH - osteophytes on patellar margins, PFOPH - osteophytes in PF joint.
*Data is adjusted for age and BMI.
†Group is too small for analysis.

The effect of each SNP often appears in presence of other alleles in certain haplotype combinations. In the present study, the analysis of the haplotypes of ADAM12 showed that the GC haplotype (rs3740199/rs1871054) has a significant protective effect by reducing the risk for development of patellofemoral osteophytes. No such association was seen for the GT haplotype. This observation suggests that rs1871054 modulates the protective effect of rs3740199 polymorphism. The protective effect of several haplotypes of four ADAM12 SNPs in relation to knee OA in a British population was reported earlier[13], although no one of the separately investigated SNPs was significantly associated with knee OA.

Progression of OA is also influenced by the genetic background[22]. Investigation of the 3-year course of knee OA in the Estonian population showed that rs3740199 SNP was associated with traits of knee OA progression measured as a change of JSN in TFJ. This association was significant in women but not in men. However, it is difficult to explain why rs3740199 SNP carries the risk of patellofemoral OA for men and seems to determine the progression rate of OA in TFJ for women. One can suppose that the process of OA in men and women differs in terms of age at onset and probably progression rate as well as location of affected joints. The prevalence of knee OA in the investigated Estonian cohort aged 32–55 years increased up to 24% during 3-year follow-up. This figure is much higher than those reported previously[12,23]. It should be mentioned that in other similar studies the mean age of participants at follow-up was over 60 (70) years. One can presume that investigation of the middle-aged population in the present study revealed the active development phase of knee OA, which resulted in the extraordinary high incidence rate. This observation is in agreement with the suggestion of the cyclic progression of knee OA[24].

The role of the ADAM12 gene and its polymorphisms in the pathogenesis of OA is not well understood. ADAM12 was recently identified as one of the four protease genes significantly over-expressed in damaged OA cartilage compared to intact one[25]. These findings support the putative role of the ADAM12 gene in cartillage destruction and OA development. Some *in vitro* studies suggest a regulatory role of ADAM12 in human bone formation and osteoclast differentiation[26]. We also found significant association between ADAM12 polymorphisms and presence of osteophytes, which confirms the possible association of this gene with bone regenerative processes.

ADAM12 is a multifunctional protein, containing pro-, metalloprotease, disintegrin and cysteine-rich domains – the components characteristic of the ADAM family. The human ADAM12 exists in two splice variants: a membrane-anchored (ADAM12m) and a shorter, secreted form (ADAM12-S) without transmembrane and cytoplasmatic domains[27]. Both, the transmembrane and the secreted forms of ADAM12 are active metalloproteases[28].

The ADAM12 protein was found to stimulate longitudinal bone growth in ADAM12-S transgenic mice by modulating chondrocyte proliferation and maturation. The metalloprotease activity of the protein was needed for that effect[29]. The recent data suggest the involving of membrane-anchored ADAM12 (ADAM-12m) in chondrocyte proliferation[30]. The ADAM12m shown to have the proteolytical activity in relation to insulin-like growth factor binding

protein 5 (IGFBP-5). It leads to enchanced bioavailability of insulin growth factor -I (IGF-I) from IGF-I-IGFBP-5 complex resulting in chondrocyte proliferation[30].

The main limitation of the present study to be mentioned is the small number of subjects with advanced stage (II + III) of knee OA. However, in a population-based study of predominantly middle-aged persons this is quite expected. In addition, several environmental risk factors (e.g., selected physical activity) contributing to knee OA were not taken into consideration. More detailed characterization of the patients' phenotype and consideration of additional risk factors in multifactorial diseases will probably disclose more significant genetic variations in development of knee OA.

Age is one of major risk factors for OA[31]. Most knee OA studies have been carried out on persons aged 60 years and older. Only a few authors have focused their studies on younger patients[32,33]. We believe that highlighting of knee OA related problems in middle-aged individuals is one of most significant advantages of our study.

In conclusion, in the present study we observed different effects of rs3740199 SNP on knee OA associated susceptibility and progression traits. First, this SNP was associated with susceptibility to OA in PFJ in middle-aged male patients. Second, rs3740199 polymorphism appears to associate progression of narrowing of the joint space, which is the result of the deterioration of the cartilage tissue mainly in TFJ in women. Regarding presence of osteophytes, the risk was more related to occurrence of bone spurs on the lateral patellar margins in PFJ. Based on these results, we suppose that the effect of the ADAM12 gene on the pathogenesis of knee OA is probably site-specific and activation of various pathogenetic mechanisms in OA seem to be remarkably gender-dependent.

Conflict of interest

There is no financial or other conflicts of interest for any of the authors related to the material contained in this manuscript.

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