

Association analysis of genotypic frequencies of matrilin-1 gene in patients with osteoarthritis

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ABSTRACT Objective

It has been suggested that genotypic variation in the gene which encodes the matrilin-1 (MATN-1) protein may be involved in the development of hip osteoarthritis (OA). We compared genotype frequencies of the MATN-1 gene (1p35) in patients with OA and controls to determine if there is any association between the MATN-1 genotype and OA.

Methods

73 OA patients and 53 controls from a rheumatology ambulatory center and a university hospital were studied. They were unrelated subjects. Controls were free of clinical OA. OA was defined according to the American College of Rheumatology criteria. The MATN-1 microsatellite in the 3'untranslated region was amplified by PCR. The size of the amplification products was determined by capillary electrophoresis in a DNA Genetic Analyzer. Genotypic distribution was compared by the χ^2 test.

Results

We identified 4 alleles according to their basepair (bp) length: A1 = 110 bp; A2 = 108 bp; A3 = 106 bp and A6 = 104 bp. Six genotypes were found, with an observed heterozygosity of 0.48. The most frequent genotype in OA and controls was A1/A1 (43.8% and 43.4%, respectively). No significant difference in genotype distribution was found between OA - even when discriminating by the affected joint - and controls.

Conclusion

We did not find any difference in the MATN-1 genotype distribution in OA patients and controls. To our knowledge, this would be the first time a MATN-1 allele of 104 bp (A6) has been identified. These results do not support a role of the MATN-1 genotypes in the occurrence of clinical OA.

Introduction

Matrilin-1 (MATN-1), originally called cartilage matrix protein (CRTM or CMP), was purified from steers by Paulsson and Heinegard in 1981 (1). This protein is considered a marker of cartilage maturation not only in chicks (2) but also in humans (3). It has been suggested that alterations in MATN-1 during endochondral bone formation

might lead to joint anatomic abnormalities, increasing the risk of developing osteoarthritis (OA) (4).

In 1992, Wang *et al.* amplified a polymorphic dinucleotide repeat of GT in the MATN-1 gene (1p35). The observed heterozygosity was 0.67 (5). This sequence is located in the 3'untranslated region (5). Five alleles have been described in different populations according to their size: A1 (110 basepairs) (bp), A2 (108 bp), A3 (106 bp), A4 (102 bp) and A5 (112 bp) (4-6). A1, A2 and A3 are common alleles, while A4 and A5 are rare (frequency < 0.5%). The MATN-1 alleles have been investigated as genetic markers in several chondrodysplasias (7) and OA (4, 8, 9). Meulenbelt *et al.* found an association between the A1 allele and hip OA in men (4). This polymorphism may be the result of a nucleotide change which by itself does not alter the translation product of the gene, but might be a marker in linkage disequilibrium with another DNA locus that indeed confers susceptibility to OA.

The aim of this study is to compare genotype frequencies of the MATN-1 gene in patients with OA and controls, to determine if there is any association between the MATN-1 genotypes and OA.

Materials and methods

Patients

The study was approved by the Bioethics Committee of the School of Medicine of the National University of Córdoba, Argentina. A total of 73 outpatients with OA and 53 controls from a rheumatology ambulatory center and a university hospital gave their informed consent to participate. They were unrelated subjects. Controls were subjects without clinical OA.

The diagnosis of OA was based on the American College of Rheumatology criteria (10). Generalized OA was defined as non-erosive hand OA, plus OA in at least two other areas. Erosive OA was diagnosed when erosions were found in at least 3 interphalangeal joints, with osteophytes, diminished joint space and subcondral sclerosis associated with inflammatory pain and/or joint deformity with a negative rheumatoid

Table I. Demographic data of controls and patients with osteoarthritis.

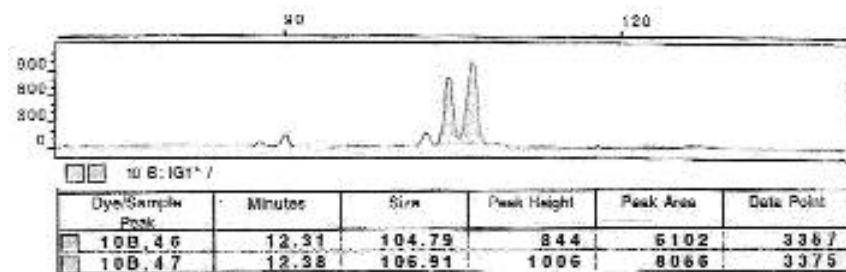
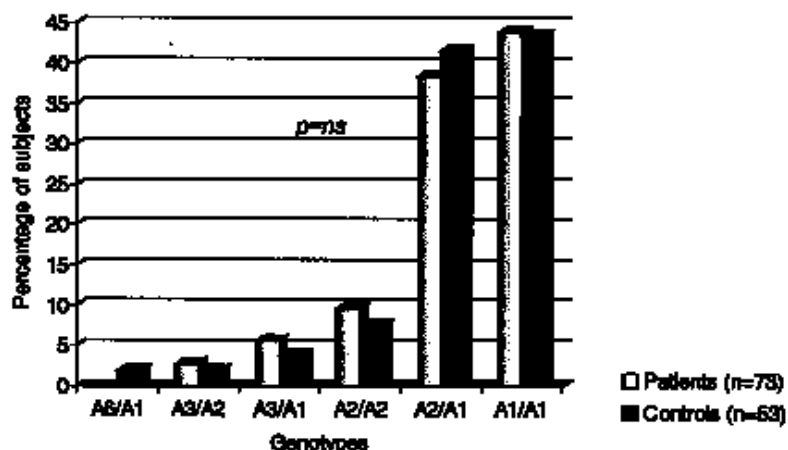
	Controls n=53	Patients n = 73
Age *, years ($\bar{X} \pm SD$)	61.2 $\pm$ 8.9	63.9 $\pm$ 10.5
BMI*§ kg/m ² ($\bar{X} \pm SD$)	26.7 $\pm$ 5.2	27.7 $\pm$ 4.5
Sex* (Female:male)	41:12	66:7

n = number of patients; § BMI: Body mass index. *Differences between groups are not significant.

Table II. Allele frequencies in patients with osteoarthritis and controls.

MATN-1 alleles (length in bp*)	A1(110 bp) n§ (%)	A2 (108 bp) n (%)	A3 (106 bp) n (%)	A6 (104 bp) n (%)
Controls ^ n of alleles =106	71 (67)	31 (29.2)	3 (2.8)	1 (0.9)
Patients ^ n of alleles =146	96 (65.8)	44 (30.1)	6 (4.1)	0 (0)

* bp: basepairs; § number. ^ Differences between groups are not significant.

**Fig. 1.** Electropherogram showing alleles A6 (104 basepairs) and A3 (106 basepairs), determined by capillary electrophoresis in a DNA Genetic Analyzer (Abi Prism® 310™).**Fig. 2.** MATN-1 genotype frequencies in patients with osteoarthritis and controls.

factor and negative antinuclear antibodies. Radiographs were assessed by two independent rheumatologists who were blinded to the clinical features. Data on age, body mass index (measured as kg/m²), ethnic origin of the participants and their parents, occupational history, other diseases, time of evolu-

tion of OA, and family history of OA were recorded. Firstly, controls were compared with all the patients. A second analysis was performed by subclassifying patients according to the affected joint (erosive and non-erosive hand OA, non-erosive generalized OA, knee, hip, lumbar and cervical spine

OA) and comparing each patient with 2 controls, who were randomly selected from the control group.

Genotyping

Genomic DNA was extracted from peripheral blood according to standard methods (11). The MATN-1 microsatellite was amplified in a PCR Pelkin Elmer Cetus DNA Thermal Cycler as described by Wang *et al.* (5). The size of the amplified fragments, which were previously labeled with 6-FAM, was determined by capillary electrophoresis in a DNA Genetic Analyzer (Abi Prism® 310™, Applied Biosynthesis).

Statistical analysis

Demographic variables, which are expressed as the mean $\pm$ standard deviation (SD), were compared using Student's t-test. Non-parametric data were analyzed using the chi-square test (²). Independence between groups and genotypic frequencies were analyzed by contingency ² and Fisher's exact tests. Correlation between radiograph reports was assessed with the Kappa coefficient (). A value higher than 0.75 was considered to indicate a good correlation, between 0.40 and 0.75 satisfactory, and less than 0.40 a poor correlation (12). Values were considered significant when $p < 0.01$. All calculated p values were 2-tailed.

Results

There were no significant differences in age, body mass index or sex distribution between patients and controls. These data are shown in Table I. Most of the patients and controls were born in Argentina (91.8% and 96.2% respectively) and were of Italian and Spanish ancestry. Forty-five patients (61.6%) had a positive family history for OA, 15 (20.5%) did not and in 13 (17.9%) the previous history was unknown. The hands and knees were the most common sites involved in the relatives of patients (24.7% and 21.9%, respectively). Regarding past occupational history, 24.7% had been teachers and 21.9% housewives. Most of the patients (61.6%) had no other rheumatic problem and 19.2% suffered osteoporosis confirmed by bone densitometry.

There was good correlation between the two X-ray observers in identifying erosive ($n = 11$) and non-erosive hand OA ($n = 26$) ($r = 0.86$ and 0.75 respectively; both $p < 0.0001$). A satisfactory correlation was found in identifying radiographic lumbar spine OA ($n = 17$; $r = 0.73$; $p = 0.003$), knee OA ($n = 26$; $r = 0.72$; $p = 0.003$), hip OA ($n = 17$; $r = 0.67$; $p = 0.002$) and cervical spine OA ($n = 22$; $r = 0.42$; $p = 0.03$).

We identified 4 alleles: A1, A2, A3 and A6. The last is a new allele of 104 bp (Fig. 1). Table II shows allele frequencies in patients and controls. Alleles were distributed in 6 genotypes: A6/A1, A3/A2, A3/A1, A2/A2, A2/A1 and A1/A1. The most frequent genotype in patients as well as in controls was the homozygous A1/A1 (43.8% and 43.4%, respectively). The overall observed heterozygosity was 0.47 in patients and 0.49 in controls. There was no significant difference in the frequency of MATN-1 genotypes between both groups (Fig. 2), nor when comparing controls with patients stratified by affected joint.

The genotype A3/A1 appeared more frequently in erosive hand OA than in controls, but the difference was not significant (A3/A1: $\chi^2 = 0.03$, Fisher's exact Test, $p = 0.10$ with 1 df).

Conclusions

There is growing evidence of a strong genetic background for hip (13), hand (14, 15), spine (14) and knee (15) OA. Current knowledge encourages the search for genetic markers in OA.

We investigated the genotypes of the MATN-1 gene after the amplification of the dinucleotide repeat in the 3' untranslated region of this gene. Four alleles and six genotypes have been observed. MATN-1 alleles A4 and A5 previously described by Wang (5) and Loughlin (6) were not identified in our group. The allele frequencies are similar to those reported in British OA patients and controls (6) and to those reported in Dutch patients not stratified by sex or the joint site involved (4). However, the allele frequencies in Dutch controls differ from Argentinian

ones. This may be due to different selection criteria used for controls: while Dutch controls were subjects without radiographic OA, ours were free from clinical OA.

For the first time an allele of 104 bp – which we have called A6 – has been identified. This may be explained by the different origins of the populations and/or by the low frequency of this allele. Our patients are predominantly of Italian and Spanish ancestry. In the global analysis, we failed to observe a significant difference of the MATN-1 genotype distribution in OA patients and controls. When analyzed by affected joints, the MATN-1 genotype A3/A1 showed an increased – but not statistically significant – frequency in erosive hand OA.

A segregation analysis concluded that OA may be transmitted as a recessive character, with residual multifactorial influence (15). We did not analyze possible external causes that may have increased susceptibility to OA.

In summary, we investigated the genotypic frequencies of the MATN-1 gene in OA patients and controls. These results do not support a role of the MATN-1 genotypes in the occurrence of clinical OA. A study with a much larger sample would nonetheless be valuable to ascertain whether there is any association between MATN-1 genotypes and OA, particularly in erosive hand OA.

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