Association of Metalloproteinase Gene Polymorphisms with Systemic Sclerosis in the European Caucasian Population

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ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is classified among the complex genetic disorders and is characterized by massive extracellular matrix deposits. These may be due to overactivation of transforming growth factor ß that may be in part a result of abnormal remodeling of extracellular matrix and microfibrils. Metalloproteinases (MMP) are a family of proteolytic enzymes, and MMP 2, 9, and 14 contribute to the degradation of microfibrils. Our aim was to determine whether polymorphisms of the *MMP2*, *MMP9*, and *MMP14* genes confer susceptibility to SSc in a large population.

Methods. A case–control study was performed in 659 SSc patients and 511 healthy matched controls from a European Caucasian population. Six Tag single-nucleotide polymorphisms (SNP) of the *MMP2* gene and 2 SNP of *MMP9* and *MMP14* genes were genotyped.

Results. All SNP were in Hardy-Weinberg equilibrium in the control population. There was no association between the *MMP2*, *MMP9*, and *MMP14* variants we investigated and SSc for allelic and genotype frequencies. No association was observed for the different subphenotypes of SSc patients.

Conclusion. Our results in a large cohort of European Caucasian SSc patients do not support that *MMP2*, *MMP9*, and *MMP14* genes are involved in the genetic background of SSc. (J Rheumatol First Release Feb 1 2010; doi:10.3899/jrheum.090973)

Key Indexing Terms:
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Systemic sclerosis (SSc) is a connective tissue disorder that belongs among the complex genetic disorders. Its hallmarks are early alterations of the microcirculation, immune system disturbances, and fibrosis. The latter corresponds to massive deposits of extracellular matrix (ECM) substances such as collagen or fibronectin[1]. Transforming growth factor ß (TGF-ß) is a leading contributor in this process[2], and accumulating evidence shows the interplay between remodeling of the ECM and activation of TGF-ß in the pathogenesis of SSc. It is now accepted that the ECM is involved in the regulation of various cytokines and growth factors including TGF-ß[3]. Indeed, fibrillin-1 (FBN-1) microfibrils, which contribute to TGF storage and regulation, may be involved in the pathogenesis of SSc[4]. *In vitro* metabolic labeling studies performed in SSc patients[5] and in healthy first-degree relatives[6] have suggested a defect of FBN-1-con-

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taining microfibrils due to excessive instability, strikingly suggesting a genetic background. Nevertheless, in a previous study we failed to demonstrate any genetic association between *FBN-1* polymorphisms and SSc[7].

Metalloproteinases (MMP) belong to a family comprising 24 members. These enzymes are able to degrade ECM participating in physiologic (wound healing, angiogenesis) and pathologic (aneurysms, cancers, arthritis) processes. MMP can degrade FBN-1 in monomer and microfibrillar forms[8]. Moreover, MMP2 and MMP9 have been implicated in the vascular damage of idiopathic aneurysms[9] and pulmonary arterial hypertension[10]. *MMP* gene single-nucleotide polymorphisms (SNP) have been found to be associated with diseases related to disorders of ECM including aneurysms (MMP9)[11].

We investigated whether *MMP2*, *MMP9*, and *MMP14* polymorphisms confer susceptibility to SSc.

MATERIALS AND METHODS

We studied 1170 unrelated subjects: 659 SSc patients classified according to LeRoy, *et al*[12] and 511 healthy matched controls. All subjects were of French European Caucasian origin, defined as all 4 grandparents being French Caucasian. The Ethics Committee of Cochin Hospital approved the study and all participants gave written informed consent.

Six *MMP2* SNP (rs −1306, rs −790, rs −735, rs1292301, rs7201, rs243849), two *MMP9* Tag SNP (rs17576, rs2274756), and two *MMP14* Tag SNP (rs743257, rs1042703) were selected using US National Center for Biotechnology Information (NCBI) data and software from the National Heart Lung and Blood Institute (http://pga.gs.washington.edu/VG2.html) was used to analyze the *MMP* genes according to HapMap data (www.hapmap.org).

Statistical analyses. Hardy-Weinberg equilibrium was investigated with a chi-square test with one degree of freedom. Power calculations were driven through an asymptomatic non-central chi-square approach and provided a power of 83% to detect association with SSc. Fisher's exact test was used to compare allele and genotype frequencies using R software, version 2.6.0. Odds ratios were calculated with the most frequent homozygous genotype or allele as reference.

RESULTS

Demographic data and disease characteristics of SSc patients are detailed in Table 1. Of 659 SSc patients, 247 (37%) had the diffuse cutaneous disease subtype and 263 (40%) had pulmonary fibrosis.

All SNP were in Hardy-Weinberg equilibrium in the control population. No significant evidence of allelic or genotypic association was detected for the *MMP2*, *MMP9*, and *MMP14* SNP (Table 2 and 3). Regarding SSc subphenotypes, intracohort comparisons failed to detect any association. The results (Table 2 and 3) include the 2 subphenotypes with the more pronounced propensity to fibrosis, that is, patients with diffuse cutaneous disease and those having fibrosing alveolitis.

DISCUSSION

In SSc, abnormal remodeling of ECM results in fibrosis characterized by excessive production and deposit of ECM substances: these disturbances are, at least in part, related to activation of TGF-ß. A growing body of evidence supports a close relationship between fibrillin-1 and TGF-ß and several studies suggest that SSc may belong among the fibrillinopathies[3]. In this context, remodeling of the microfibrillar network in SSc could contribute to TGF-ß overactivation. In SSc, it seems that there is a striking decrease of the microfibrillar network in both affected and nonaffected areas of skin in early diffuse SSc disease (data not published), which could be secondary to an excessive instability of SSc microfibrils[13]. This might be due to excessive constitutional proteolysis. Functional polymorphisms of MMP genes, which encode for ECM proteolytic enzymes, may increase the proteolytic activity of MMP and subsequently the degradation of the ECM. However, our study failed to detect any association between *MMP2*, *MMP9*, and *MMP14* SNP and SSc. A phenotype-genotype correlation study also failed to detect any association.

Methodological limitations of genetic studies must always be considered. Appropriate sample sizes for case and control cohorts are critical to provide sufficient statistical power. In this study, the large sample size provided adequate power (83%). Moreover, the genetic background of the study population should be as homogeneous as possible to limit bias by population stratification; ethnicity was taken into account to avoid this bias and we focused on European Caucasian individuals. Finally, allelic and genotypic frequencies in our controls were in agreement with those reported for European Caucasian populations (NCBI data). These factors favor the validity of our results, and support the lack of association between SSc and the MMP genes investigated.

MMP-2, 9, and 14 were of particular interest for several reasons: (1) previous studies suggested that MMP-2, 9, and 14 play a key role in the turnover of fibrillin-1 molecule and microfibrils[8]; (2) MMP-2 and 9 are largely implicated in vascular remodeling and pathologies[9,10]; (3) some authors have suggested that polymorphism of the *MMP14* gene may

Table 1. Characteristics of the patients with systemic sclerosis.

Patients	SSc Cohort, n = 659
Age, yrs ± SD	59 ± 11
Female, n (%)	574 (87)
Disease duration, yrs ± SD	11 ± 8
Diffuse cutaneous subtype, n (%)	247 (37)
Pulmonary fibrosis, n (%)	263 (40)
DLCO/VA < 75%, n (%)	291 (44)
Pulmonary arterial hypertension (defined by right heart catheterization, n (%)	51 (8)
Digital ulcerations, n (%)	199 (30)
Positive anti-topoisomerase I antibodies, n (%)	163 (25)
Positive anti-centromere antibodies, n (%)	270 (41)

DLCO/VA: diffusing capacity for carbon monoxide divided by alveolar volume.

Table 2. Frequencies of *MMP2* alleles and genotypes in SSc, in the diffuse cutaneous subset, in SSc patients with fibrosing alveolitis and controls. Data are no. (%).

Feature		SSc	dcSSc	FA	Controls	p*
rs-1306 prom						
Allele	C	1364 (78)	467 (78)	542 (78)	1544 (76)	0.24
	T	392 (22)	129 (22)	156 (22)	486 (24)	
Genotypes	CC	535 (61)	187 (63)	217 (62)	596 (59)	0.3
	TC	294 (33)	93 (31)	108 (31)	352 (35)	
	TT	49 (6)	18 (6)	24 (7)	67 (6)	
rs-790 prom						
Allele	T	1341 (78)	467 (79)	533 (78)	1529 (76)	0.22
	G	377 (22)	113 (21)	147 (22)	720 (24)	
Genotypes	TT	528 (61)	183 (63)	214 (63)	592 (59)	0.28
	TG	285 (33)	91 (31)	105 (31)	345 (34)	
	GG	46 (6)	16 (6)	21 (6)	64 (7)	
rs-735 prom						
Allele	C	1518 (87)	521 (89)	613 (89)	1752 (88)	0.62
	T	216 (13)	67 (11)	73 (11)	236 (12)	
Genotypes	CC	669 (77)	232 (79)	274 (80)	769 (77)	0.35
	TC	188 (22)	57 (19)	65 (19)	214 (22)	
	TT	14 (1)	5 (2)	4 (1)	11 (1)	
rs1292301						
Allele	C	1135 (65)	399 (68)	460 (65)	1326 (65)	0.84
	T	611 (35)	191 (32)	232 (35)	704 (35)	
Genotypes	CC	372 (43)	136 (46)	153 (44)	436 (43)	0.83
	TC	391 (45)	127 (43)	154 (44)	454 (45)	
	TT	110 (12)	32 (11)	39 (12)	125 (12)	
rs7201						
Allele	A	972 (56)	330 (57)	385 (56)	1090 (55)	0.58
	C	760 (44)	250 (43)	299 (44)	884 (45)	
Genotypes	AA	285 (33)	101 (35)	111 (32)	313 (32)	0.61
	AC	402 (46)	128 (44)	163 (48)	464 (47)	
	CC	179 (21)	61 (21)	68 (20)	210 (21)	
rs243849						
Allele	C	1445 (83)	488 (83)	585 (85)	1650 (83)	0.77
	T	299 (17)	100 (17)	103 (15)	350 (17)	
Genotypes	CC	602 (69)	205 (70)	251 (73)	686 (69)	0.74
	TC	241 (28)	78 (27)	83 (24)	278 (28)	
	TT	29 (3)	11 (3)	10 (3)	36 (3)	

* Comparison of frequencies of minor allele and homozygous genotype between SSc and controls. dcSSc: diffuse cutaneous systemic sclerosis; FA: SSc patients with fibrosing alveolitis.

be responsible for the upregulation of protein activity leading to degradation of the extracellular matrix[15]. Nevertheless, other variants and other MMP genes or partners should be investigated to assess these pathways in SSc pathogenesis.

We observed that the *MMP2*, *MMP9*, and *MMP14* genes did not contribute to the genetic background of SSc in a French European Caucasian population. Further functional investigations are needed to determine the potential roles of MMP-2, 9, and 14 in the pathogenesis of SSc.

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Table 3. Frequencies of *MMP9* and *MMP14* alleles and genotypes in SSc, in the diffuse cutaneous subset, in SSc patients with fibrosing alveolitis and controls. Data are no. (%).

Feature		SSc	dcSSc	FA	Controls	p*
MMP9 gene						
rs17576						
Allele	A	1131 (65)	372 (63)	438 (64)	1345 (66)	0.71
	G	603 (35)	216 (37)	250 (36)	699 (34)	
Genotypes	AA	390 (45)	129 (44)	147 (43)	444 (43)	0.24
	GA	351 (40)	114 (39)	114 (42)	457 (45)	
	GG	126 (15)	51 (17)	53 (15)	121 (12)	
rs2274756						
Allele	G	1512 (86)	510 (86)	583 (84)	1756 (86)	0.77
	A	246 (14)	84 (14)	109 (16)	278 (14)	
Genotypes	GG	646 (73)	221 (74)	245 (71)	754 (74)	0.98
	AG	220 (25)	68 (23)	93 (27)	248 (24)	
	AA	13 (2)	8 (3)	8 (2)	15 (2)	
MMP14 gene						
rs743257						
Allele	C	812 (46)	303 (51)	339 (49)	946 (47)	0.65
	T	944 (54)	293 (49)	357 (51)	1068 (53)	
Genotypes	CC	194 (22)	83 (28)	89 (26)	225 (22)	0.68
	TC	424 (54)	137 (46)	161 (46)	496 (50)	
	TT	260 (24)	78 (26)	98 (28)	286 (28)	
rs1042703						
Allele	T	1457 (85)	487 (84)	567 (84)	1711 (84)	0.77
	C	263 (15)	93 (16)	111 (16)	317 (16)	
Genotypes	TT	619 (72)	205 (71)	235 (69)	722 (71)	0.93
	CT	219 (25)	77 (26)	97 (29)	267 (26)	
	CC	22 (3)	8 (3)	7 (2)	25 (3)	

* Comparison of frequencies of minor allele and homozygous genotype between SSc and controls. dcSSc: diffuse cutaneous systemic sclerosis; FA: SSc patients with fibrosing alveolitis.

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