Correlation between angiotensin-converting-enzyme 2 gene polymorphisms and systemic sclerosis

B. Miziołek[1], C. Kruszniewska-Rajs[2], J. Gola[2], M. Bultrowicz[3], J. Miszczyk[4], R. Pieczyrak[3], A. Frątczak[1], K. Polak[1], E. Kucharz[3], B. Bergler-Czop[1]

[1]Department of Dermatology, School of Medicine in Katowice, Medical University of Silesia, Katowice; [2]Department of Molecular Biology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice; [3]Department of Internal Medicine, Rheumatology and Clinical Immunology, School of Medicine in Katowice, Medical University of Silesia, Katowice; [4]Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland.

Abstract

Objective

Systemic sclerosis (SSc) is a disease with cardiovascular impairment and polymorphisms of the gene coding of angiotensin-converting-enzyme 2 (ACE2) may account for its development. Three single nucleotide polymorphisms of ACE2 (C>G rs879922, G>A rs2285666 and A>G rs1978124) were found to increase the risk for development of arterial hypertension (AH) and cardiovascular (CVS) diseases in different ethnicities. We investigated associations of polymorphisms rs879922, rs2285666 and rs1978124 with the development of SSc.

Methods

Genomic DNA was isolated from whole blood. Restriction-fragment-length polymorphism was used for genotyping of rs1978124, while detection of rs879922 and rs2285666 was based on TaqMan SNP Genotyping Assay. Serum level of ACE2 was assayed with commercially available ELISA test.

Results

81 SSc patients (60 women, 21 men) were enrolled. Allele C of rs879922 polymorphism was associated with significantly greater risk for development of AH (OR=2.5, p=0.018), but less frequent joint involvement. A strong tendency to earlier onset of Raynaud's phenomenon and SSc was seen in carriers of allele A of rs2285666 polymorphism. They had lower risk for development of any CVS disease (RR=0.4, p=0.051) and tendency to less frequent gastrointestinal involvement. Women with genotype AG of rs1978124 polymorphism had significantly more frequent digital tip ulcers and lower serum level of ACE2.

Conclusion

Polymorphisms of ACE2 may account for the development of AH and CVS disorders in SSc patients. Strong tendencies to more frequent occurrence of disease specific characteristics distinct to macrovascular involvement will require further studies evaluating significance of ACE2 polymorphisms in SSc.

Key words

systemic sclerosis, angiotensin-converting-enzyme 2, ACE2, angiotensin, polymorphisms

Bartosz Miziołek, MD, PhD
(ORCID: 0000-0001-8388-4419)
Celina Kruszniewska-Rajs, PhD
(0000-0002-2504-6289)
Joanna Gola, PhD
(ORCID: 0000-0002-3089-0409)
Monika Bultrowicz, MD
Justyna Miszczyk, PhD
(ORCID: 0000-0001-7226-1750)
Robert Pieczyrak, MD, PhD
(0000-0003-1882-0210)
Aleksandra Frątczak, MD
(ORCID: 0000-0001-9047-7483)
Karina Polak, MD
(ORCID: 0000-0001-7785-5427)
Eugeniusz Kucharz, MD, PhD
(ORCID: 0000-0001-5571-8114)
Beata Bergler-Czop, MD, PhD
(ORCID: 0000-0003-3788-5984)

Please address correspondence to:
Bartosz Miziołek
Department of Dermatology,
Medical University of Silesia,
School of Medicine in Katowice,
20/24 Francuska St.,
40067 Katowice, Silesia, Poland.
E-mail: bmiziolek@gmail.com

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Introduction

Systemic sclerosis is a disease with cardiovascular impairment, but mechanisms responsible for its development remain under ongoing investigations (1). One of them appears to be a dysregulation in functioning of the renin-angiotensin-system and previously a significantly reduced plasma level of angiotensin-(1-7) was found in patients with SSc producing an unfavourable ratio of angiotensin-(1-7) (Ang-(1-7)) to angiotensin II (Ang II) in them (2, 3). The balance between the activity of these antagonistic substances is regulated mainly by angiotensin-covertingenzyme-2 (ACE2), which degrades Ang II to Ang-(1-7) (4). There were identified autoantibodies aimed against ACE2 (anti-ACE2) in patients with severe constrictive vasculopathy in different connective tissue diseases which correlated with plasma level of Ang-(1-7) under detection level, but these antibodies were found only in a small percentage of SSc patients (5, 6). Probably there may exist other factors influencing on the activity of ACE2 and possible one is polymorphism of the gene coding the enzyme, but it has not been investigated in SSc settings yet.

The aim of this study was to investigate correlations between three polymorphisms: rs879922, rs2285666 and rs1978124, and development of SSc. All these single nucleotide polymorphisms (SNPs) of the gene coding ACE2 were found to increase the risk for development of arterial hypertension (AH) and cardiovascular diseases (CVS) in different ethnicities (7).

Material and methods

Study group

Patients were enrolled from the Regional Scleroderma Center (RSC) in Katowice: Department of Dermatology in Katowice and Department of Internal Medicine, Rheumatology and Clinical Immunology at the Medical University of Silesia in Katowice, Poland. All of them met classification criteria for SSc established by the European League Against Rheumatism and the American College of Rheumatology in 2013. Prior to enrolment they were provided with a written informed consent form, and the study was earlier approved by the local ethical committee at the Medical University of Silesia in Katowice, Poland (PCN/CBN/0052/KB1/29/22). Medical records were reviewed for each of patients to extract data on the subtype of the disease (limited, lcSSc or diffuse, dcSSc), an immune profile of antinuclear antibodies (ANA), an onset of Raynaud's phenomenon (RP) and the first symptom of SSc other than RP, any presence of trophic lesions on hands, an involvement of internal organs and any coexistence of cardiovascular disorders.

Identification of ANA, using the commercially available immunoblot test kits, and detailed examination of patient's hands to detect signs of active (digital ulcers) or previous (pitting scars) trophic lesions constitute elements of routine follow-up visits for every patient at our RSC. ANA were categorised into anticentromeric (ACA), anti-topoisomerase I (anti-Scl70) and other, less frequently detected antibodies (anti-polymerase III RNA, anti-Ku, anti-Th/To). An evaluation of internal organ involvement included development of interstitial lung disease (ILD) as well as kidney, gastrointestinal and joint involvement. ILD was recognised based on the routinely performed high-resolution CT scans of the chest and pulmonary function tests. Kidney involvement (KI) included decreased glomerular filtration rate below 60 ml/min and/or albuminuria ≥30 g daily and/or urine sediment abnormalities (dysmorphic erythrocytes, urine casts) observed for at least 3 months. Joint involvement (JI) was defined clinically when a given patient suffered from arthralgia. Gastrointestinal involvement (GI) was evaluated with panendoscopy or barium contrast x-ray of the oesophagus and the stomach. Early onset of RP ad SSc was classified when it commenced before 50 years of age.

To characterise cardiovascular involvement, medical data were extracted on the presence of pulmonary arterial hypertension (PAH), AH, cardiac arrythmia, episodes of previous myocardial infarction (MI) and/or cerebral stroke (CS). PAH was recognised via right heart catheterisation in the previously

selected patients using the DETECT algorithm (1, 8). AH was recognised based on guidelines established by European Society of Cardiology and the European Society of Hypertension in 2018 (9). Each diagnosis of cardiac arrythmia was recorded on electrocardiograms and/or earlier reported by a consultant cardiologist. Episodes of MI or CS to be included in the analysis had to be confirmed by medical documentation of their treatment from units specialised at intensive care, cardiology or neurology. Cardiovascular disorders were grouped in the final analysis as following: AH, cardiac arrythmia, PAH, and any cardiovascular disease. The last group included the presence of AH, cardiac arrythmia, PAH or previous episode of MI/CS.

Blood sampling and laboratory measurements

Two blood samples were taken from the antecubital vein. The first tube of the whole blood for later genotyping was stored at -80°C. The second blood sample was collected in a clot activator tube and centrifuged at 1996 RCF for 15 min to obtain serum sample, which then was stored at -80°C until ACE2 level was measured.

Genomic DNA was isolated from whole blood using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Gdansk, Poland). Concentration of DNA was quantified using spectrophotometer MaestroNano MN-913 (MaestroGen Inc, USA). Genotyping of rs1978124 was performed according to protocols for restriction-fragment-length polymorphism (RFLP), while detection of rs879922 and rs2285666 was based on TaqMan SNP Genotyping Assay. The details of genotyping are described in the Supplementary file. The ACE2 Serum level was assayed with the use of a commercially available ELISA test obtained from Thermo-Fisher (no. EH489RB, Waltham, USA). Analytical sensitivity of the kit was 0.025 ng/ml with a detection range: 0.027-20 ng/ml. Absorbance at 450 nm was read by BioTek Epoch Spectrophotometer (Agilent, Santa Clara, USA) with dedicated Gen5 Data Analysis Software.

Table I. Clinical characteristics of patients.

	women (n=60)	men (n=21)	*p*-value
Age (years)	54 ± 14	57 ± 12	0.47*
SSc subtype			
- limited	53 (88%)	11 (52%)	0.001***
- diffuse	7 (12%)	10 (48%)	
Antinuclear antibodies			
- ACA	17 (28%)	2 (9%)	<0.001**
- Scl70	33 (55%)	5 (24%)	
- other	10 (17%)	14 (67%)	
Onset of Raynaud's phenomenon (RP)			
- early	42 (70%)	11 (52%)	0.14**
- late	18 (30%)	10 (48%)	
Onset of SSc (from first non-RP sign)			
- early	37 (62%)	9 (43%)	0.13**
- late	23 (38%)	12 (57%)	
Interstitial lung disease	32 (53%)	16 (76%)	0.076**
Trophic lesions	29 (48%)	15 (71%)	0.07**
Kidney involvement	9 (15%)	7 (33%)	0.11***
Any CVS disease	41 (68%)	12 (57%)	0.43**
Cardiac arrythmia	23 (38%)	9 (43%)	0.8**
Arterial hypertension	26 (43%)	7 (33%)	0.42**
Pulmonary arterial hypertension	4 (7%)	1 (5%)	1.0***
Gastrointestinal involvement	34 (57%)	8 (38%)	0.14**
Joint involvement	25 (42%)	5 (24%)	0.14**
Serum level of ACE2 (ng/ml)	0.087	0.048	0.008****

*Student t-test; **χ^2 test; ***Fisher's exact test; ****Mann-Whitney U-test.

Statistical analysis

The correlation between results of genotyping and characteristics of SSc patients was analysed using χ^2 test, and Fisher's exact test, when the number of subjects in a subgroup was below five. A deviation from Hardy-Weinberg equilibrium was checked using χ^2 test and Yate's correction. The corresponding differences in serum level of ACE2 were checked with the use of Mann-Whitney U-test. A *p*-value below 0.05 had statistical significance. Statistical analysis was performed using PQStat Software (v. 1.8.0.476, PQStat Software, Poznań, Poland).

Results

There were enrolled eighty-one patients with SSc between April and December 2022. All of them were Caucasians and their detailed characteristics are included in Table I. The distribution of genotypes remained in Hardy-Weinberg equilibrium for all polymorphisms despite of the small study sample (Table II).

Carrying of C allele of rs879922 polymorphism was associated with significantly greater risk for development of essential AH (OR=2.5 (1.09-5.85) *p*=0.018), but with the tendency to less frequent development of JI (*p*=0.053) (Table III). Most women with CC genotype of rs879922 polymorphism developed AH, whereas most patients with GG genotype had JI (Table IV).

There occurred a strong tendency for earlier onset of both RP (*p*=0.056) and first non-RP symptom of SSc (*p*=0.057) in carriers of allele A of rs2285666 polymorphism, but these patients had lower risk for the development of any CVS disease (RR=0.4 (0.14–1.11) *p*=0.05) and showed tendency for less frequent GI (*p*=0.06) (Table III). There occurred a tendency (*p*=0.086) to more frequent development of dcSSc in subjects having GA genotype of rs2285666 polymorphism when compared to those with GG genotype, but the strong tendency to more frequent KI was observed in patients with GG genotype (*p*=0.056). Essential AH, CVS diseases and GI were significantly more frequently reported in subjects with GG genotype (Table IV). Due to a small number of patients with AA genotype of rs2285666 polymorphism and GG genotype of rs1978124 polymorphism, these both genotypes were not included

Table II. Distribution of alleles and genotypes.

	Alleles			*p*-value	Genotypes		*p*-value
		women (n=60)	men (n=21)		women		
rs879922	C	55	9	0.8	CC	12	0.95
	G	65	12		CG	31	
					GG	17	
rs2285666	G	95	19	0.36*	GG	37	0.98**
	A	25	2		GA	21	
					AA	2	
rs1978124	A	94	16	0.78	AA	35	0.56**
	G	26	5		AG	24	
					GG	1	

*Fisher's exact test; **Yates χ^2 test.

in the main statistical analysis, but they are seen in the Supplementary file.

No significant tendencies were observed for allele G and A of rs1978124 polymorphism (Table III), but in women, genotype AG of rs1978124 polymorphism was associated with significantly more frequent development of DU and these patients had significantly lower serum level of ACE2 than those with AA genotype (Table IV).

Discussion

The gene coding of ACE2 is highly polymorphic, and several intronic variants of the gene coding ACE2 were previously analysed mainly in the context of CVS disorders. Three polymorphisms, namely rs2285666, rs1978124 and rs879922, emerged as risk factors for the development of AH in different ethnicities (7). They include SNPs G->A at nucleotide 8790 of intron 3, A->G at nucleotide 1075 of intron 1, and C->G at nucleotide 28330 of intron 11, respectively (10). The significance of these polymorphisms for the development of SSc patients has not been analysed yet and our research may serve as a pilot study. The location of the gene for ACE2 on X chromosome allows for the analysis of genotypes only in women.

This study highlighted primarily the correlation between polymorphisms of the gene for ACE2 and the development of macrovascular disease, but, to a lesser extent, the microvascular involvement. Allele C of rs879922 polymorphism was found in this study to be associated with the development of essential AH, which remains in accordance with previous observations in Chinese studies (11-13) but contrary to the German study, which did not show the correlation between allele C and development of AH (14).

Lower risk for the development of any CVS disease in carries of allele A of rs2285666 (G8790A) polymorphism may seem to be surprising, since the allele was previously reported in several Chinese studies to be associated with increased risk for the development of AH (12, 15, 16), carotid arteriosclerosis ≥50% (11), myocardial infarction in women (17) and cerebral stroke in patients with DM2 (18). Other studies showed however G8790A polymorphism not to be associated with essential AH among Australian people of white Anglo-Celtic origin (10) and German population (14). A large meta-analysis involving 11,051 subjects identified G8790A polymorphism to be even a protective factor against AH in non-Han Chinese males (19). Allele A of rs2285666 polymorphism was linked to a significantly reduced risk of CVS death in European women, whereas the allele was identified to be the risk factor for CVS death in Asian population (20). Reports on a protective role of allele A of rs228566 in European women are consistent with our observations on less frequent CVS disorders and KI in SSc patients carrying this allele and SSc women with GA genotype.

The third of analysed polymorphisms among our SSc population was rs1978124 (A1075G). The data on the association between this polymorphism and essential AH remains inconclusive. The Chinese study identified almost 2-fold higher susceptibility to development of essential AH (15), but the other one showed lack of such association (11). The study on Australian inhabitants of white Anglo-Celtic origin did not show greater risk for development of AH associated with rs1978124 polymorphism, but male carriers of G allele had higher diastolic blood pressure (10). Although no association of rs1978124 polymorphism with development of coronary heart disease observed in Chinese population, the risk of myocardial infarction was noticed to be greater in women with AA genotype (17).

Polymorphisms are known to show distinct phenotypes regarding to ethnicity. The dependence on ethnicity was previously seen at analysis of insertion/deletion (I/D) polymorphism of the gene coding for ACE. A deletion of 287-base pair Alu repeat within intron 16 was found to be a potential susceptibility factor for the development of SSc only among Italian people (21-23) but not in neighbour Mediterranean population of Greek, whose frequency of the polymorphism was not greater in SSc patients when compared to healthy controls (21). Neither the multi-ethnic American study (24) nor the Korean (25) or French ones involving European Caucasians (26) confirmed the correlation between ACE I/D polymorphism and greater risk for the development of SSc.

The only one of the analysed ACE2 variants which showed herein any association with serum ACE2 level was rs1978124 polymorphism. It was also the only one polymorphism in our study which revealed the correlation between

Table III. Dependence of disease characteristics on alleles.

	rs879922			rs2285666			rs1978124		
	C	G	*p*-value	G	A	*p*-value	A	G	*p*-value
Disease SSc									
- limited	44 (85%)	49 (82%)	0.68	62 (81%)	18 (72%)	0.37	60 (80%)	26 (87%)	0.42
- diffuse	8 (15%)	11 (18%)		15 (19%)	7 (28%)		15 (20%)	4 (13%)	
Antinuclear antibodies									
- ACA	15 (29%)	12 (20%)	0.55	19 (25%)	6 (24%)	0.96	18 (24%)	6 (20%)	0.55
- Scl70	29 (56%)	38 (63%)		44 (57%)	15 (60%)		44 (59%)	16 (53%)	
- other	8 (15%)	10 (17%)		14 (18%)	4 (16%)		13 (17%)	8 (27%)	
Onset of Raynaud's phenomenon (RP)									
- early	33 (63%)	42 (70%)	0.46	49 (64%)	21 (84%)	0.056	50 (67%)	19 (63%)	0.75
- late	19 (37%)	18 (30%)		28 (36%)	4 (16%)		25 (33%)	11 (37%)	
Onset of SSc (from first non-RP sign)									
- early	28 (54%)	37 (62%)	0.4	42 (55%)	19 (76%)	0.057	44 (59%)	15 (50%)	0.42
- late	24 (46%)	23 (38%)		35 (45%)	6 (24%)		31 (41%)	15 (50%)	
Interstitial lung disease	27 (52%)	36 (60%)	0.39	45 (58%)	14 (56%)	0.83	43 (57%)	20 (67%)	0.38
Active or previous trophic lesions	26 (50%)	34 (57%)	0.48	42 (55%)	12 (48%)	0.57	41 (55%)	19 (63%)	0.42
Kidney involvement	9 (17%)	11 (18%)	0.89	15 (19%)	2 (8%)	0.23*	15 (20%)	4 (13%)	0.42
Any cardiovascular disease	37 (71%)	36 (60%)	0.22	51 (66%)	11 (44%)	0.048	49 (65%)	19 (63%)	0.85
				OR=0.4 (0.14-1.11) p=0.05					
Cardiac arrhythmia	19 (37%)	25 (42%)	0.58	31 (40%)	10 (40%	0.98	29 (39%)	11 (37%)	0.85
Arterial hypertension	28 (54%)	19 (32%)	0.018	32 (42%)	6 (24%)	0.11	31 (41%)	12 (40%)	0.9
	OR=2.5 (1.09-5.85) *p*=0.018								
Pulmonary arterial hypertension	1 (2%)	5 (8%)	0.21*	4 (5%)	1 (4%)	1.0*	4 (5%)	2 (7%)	1.0*
Gastrointestinal involvement	27 (52%)	32 (53%)	0.88	41 (53%)	8 (32%)	0.06	38 (51%)	19 (63%)	0.24
Joint involvement	15 (29%)	28 (47%)	0.053	28 (36%)	8 (32%)	0.69	27 (36%)	14 (47%)	0.31
ACE2 (ng/ml)	0.081	0.069	0.14	0.0726	0.0745	0.71	0.073	0.039	0.1

*Fisher's exact test; OR: odds ratio.

Table IV. Dependence of disease characteristics on genotypes.

	rs879922				rs2285666			rs1978124		
	CC	CG	GG	*p*-value	GG	GA	*p*-value	AA	AG	*p*-value
SSc subtype										
- limited	11 (92%)	29 (94%)	13 (76%)	0.24*	35 (95%)	16 (76%)	0.086*	30 (86%)	22 (92%)	0.69*
- diffuse	1 (8%)	2 (6%)	4 (24%)		2 (5%)	5 (24%)		5 (14%)	2 (8%)	
Antinuclear antibodies										
- ACA	6 (50%)	8 (26%)	3 (18%)	0.16*	11 (30%)	6 (29%)	0.97	12 (34%)	5 (21%)	0.3
- Scl70	4 (33%)	20 (64%)	9 (53%)		20 (54%)	12 (57%)		19 (54%)	13 (54%)	
- other	2 (17%)	3 (10%)	5 (29%)		6 (16%)	3 (14%)		4 (12%)	6 (25%)	
Onset of Raynaud's phenomenon										
- early	7 (58%)	22 (71%)	13 (76%)	0.57	23 (49%)	17 (81%)	0.013	26 (74%)	16 (67%)	0.53
- late	5 (42%)	9 (29%)	4 (24%)		24 (51%)	4 (19%)		9 (26%)	8 (33%)	
Onset of SSc (from first non-RP sign)										
- early	6 (50%)	19 (61%)	12 (71%)	0.53	20 (54%)	15 (71)	0.19	24 (69%)	13 (54%)	0.26
- late	6 (50%)	12 (39%)	5 (29%)		17 (46%)	6 (29%)		11 (31%)	11 (46%)	
Interstitial lung disease	5 (42%)	15 (48%)	12 (71%)	0.22	19 (51%)	11 (52%)	0.93	16 (46%)	15 (62%)	0.2
Active or previous trophic lesions	4 (33%)	16 (52%)	9 (53%)	0.51	19 (51%)	10 (48%)	0.78	13 (37%)	16 (67%)	0.025
Kidney involvement	5 (24%)	4 (13%)	7 (24%)	0.48	14 (25%)	1 (5%)	0.056*			
Any cardiovascular disease	10 (83%)	20 (65%)	9 (53%)	0.24	29 (78%)	9 (43%)	0.006	23 (66%)	15 (62%)	0.8
Cardiac arrhythmia	3 (25%)	12 (39%)	6 (35%)	0.7	12 (32%)	9 (43%)	0.43	13 (37%)	8 (33%)	0.76
Arterial hypertension	9 (75%)	14 (45%)	3 (18%)	0.009	20 (54%)	5 (24%)	0.025	15 (43%)	10 (42%)	0.93
Pulmonary arterial hypertension	0	1 (3%)	2 (12%)	0.29*	2 (5%)	0	0.53*	2 (6%)	1 (4%)	1.0*
Gastrointestinal involvement	7 (58%)	17 (55%)	6 (35%)	0.35	23 (62%)	7 (33%)	0.034	15 (43%)	15 (62%)	0.14
Joint involvement	1 (8%)	13 (42%)	10 (59%)	0.02	17 (46%)	6 (29%)	0.19	11 (31%)	12 (50%)	0.15
ACE2 (ng/ml)	0.107	0.084	0.049	0.14**	0.078	0.074	0.72***	0.09	0.031	0.005***

*Fisher's exact test; **ANOVA Kruskal-Wallis; ***Mann-Whitney U-test.

genotypes and microvascular involvement. Genotype AG was found to be associated with significantly more frequent development of trophic lesions and significantly lower serum level of ACE2 than AA genotype. One may only speculate the dependence of microvascular disease on ACE2 serum level, but the analysis did not include GG genotype due to a low number of enrolled patients. The significant activation of the RAS (11) and lower expression of ACE2 were earlier found in patients carrying C allele of rs879922 polymorphism (12), but the other study showed neither level of circulating ACE2 nor one of angiotensin-(1-7) to be related to rs879922 polymorphism (27).

Our study showed an indirect influence of rs2285666 polymorphism on the microvascular involvement. There was seen a strong tendency to earlier onset of RP and SSc, but to evaluate the significance of allele A for the development of microvasculopathy a larger study group is required. The study also revealed tendencies to more frequent development of JI in patients carrying G allele of rs879922 and association of GI with G allele of rs2285666 variant of the ACE2. Both tendencies are difficult for interpretation since no previous studies on ACE2 polymorphisms analysed polymorphisms of ACE2 in SSc. Although both disease complications seem to remain irrespective to the gene coding enzyme regulating the CVS seems, the recent paper evaluating the incidence of AH in patients with SSc showed its significantly more frequent coexistence with oesophageal involvement (28). Finally, the statistical tendency to more frequent development of dcSSc in case of GA genotype of rs2285666 polymorphism suggests allele A may serve as a poor prognostic factor, but once again, the larger number of patients need to be enrolled to evaluate such correlation.

Although this study is probably the first attempt to assess associations between polymorphisms of the gene coding of ACE2 and the development of SSc, we are aware of its severe limitations. The first of them was enrolment of patients from the only single RSC which limited the size of study sample. The small number of patients did not allow for the comparison of possible three genotypes in case of rs2285666 and rs1978124 polymorphisms. Further studies should also involve a greater number of patients with PAH since current results remain inconclusive, because only a few subjects suffering from this disease complication were enrolled. The second severe limitation was the analysis of only three polymorphisms, whereas at least several other variants of the gene were reported. One may speculate a lack of healthy controls involved to the study, but we decided to refer results to earlier studies investigating prevalence and associations between polymorphism of the gene coding ACE2 and arterial hypertension in different ethnicities, including neighbour German population.

In conclusion, polymorphisms of the gene coding ACE2 may account for the development of AH and CVS disorders in SSc patients. Strong tendencies to more frequent occurrence of disease specific characteristics distinct to macrovascular involvement (earlier onset of RP and first non-RP sign, development of dcSSc, trophic lesions, GI, JI) show the need for further studies evaluating significance of ACE2 polymorphisms for the development of SSc.

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