

## Supplementary material

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*Michalska-Jakubus M, Rusek M, Kowal M, Krasowska D. Preliminary observations on ANGPT1 and ANGPT2 polymorphisms in systemic sclerosis: ANGPT2 rs2442598 and rs3739390 are associated with disease susceptibility and diffuse disease subtype. Pol Arch Intern Med. 2021; 131: 16121. doi:10.20452/pamw.16121*

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Table S1. Primers used for the genotyping of Angiopoietin 2 gene (ANGPT2) based on He et al. [21].

Gene	SNP	Direction	PCR primer	Product length, bp
ANGPT2	rs3739390	Forward	AAGAGCGGCAGTTGTCCAT	385
		Reverse	GTTCTTCCCCTGCAATCTGAC	
ANGPT2	rs2442598	Forward	GCAAACCATGCAGCTAGAAG	366
		Reverse	CTGCACCGAACTGCTCTCA	

Abbreviations: ANGPT2, angiopoietin 2 gene; bp, base pair; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Table S2. Results of Hardy-Weinberg equilibrium test for genotypes distribution of investigated single nucleotide polymorphisms in systemic sclerosis patients and healthy controls.

SNP	HWE			
	HCs		SSc patients	
	$\chi^2$	P value	$\chi^2$	P value
ANGPT1 rs2507800	0.022	0.989 <sup>a</sup>	0.995	0.61 <sup>a</sup>
ANGPT2 rs3739390	1.230	0.541 <sup>a</sup>	0.228	0.89 <sup>a</sup>
ANGPT2 rs2442598	45.43	<0.001 <sup>b</sup>	46.95	<0.001 <sup>b</sup>

<sup>a</sup> chi-squared test with Yates' correction

<sup>b</sup> chi-squared test without correction

Abbreviations: ANGPT1, angiotensinogen converting enzyme 1 gene; ANGPT2, angiotensinogen converting enzyme 2 gene; HCs, healthy controls; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; SSc, systemic sclerosis

Table S3. Irrelevant associations between rs2507800 of ANGPT1 and rs3739390 of ANGPT2 gene and risk of selected clinical variables among systemic sclerosis patients.

SNPs	Clinical variable	Allele frequency, n (%)				Genotype frequency, n (%)								
						Additive model				Dominant model			Recessive model	
ANGPT1 rs2507800		T	A	<i>P</i> <sup>a</sup>	OR	TT	AT	AA	OR	OR	AA+AT	OR	TT+AT	OR
					(95%CI)				[TT/AT]	[TT/AA]		[TT/AA +AT]		[AA/TT +AT]
								(95%CI)	(95%CI)		(95%CI)		(95%CI)	
ILD (-)		8	6	0.97	1.00 (ref.)	2	4	1	1.00 (ref.)	1.00 (ref.)	5 (71)	1.00 (ref.)	6 (86)	1.00 (ref.)
		(57)	(43)			(29)	(57)	(14)						
ILD (+)		39	25	0.97	0.837	11	17	4	0.791	0.666	21 (66)	0.766	28 (87)	1.291
		(61)	(39)		(0.257- 2.725)	(34)	(53)	(13)	(0.117- 5.350)	(0.038- 11.602)		(0.122- 4.816)		(0.113- 14.713)
ACA		24	12	0.28	1.00 (ref.)	8	8	2	1.00 (ref.)	1.00 (ref.)	10 (45.5)	1.00 (ref.)	16 (89)	1.00 (ref.)
		(67)	(33)			(44.5)	(44.5)	(11)						
Scl-70		23	19		1.652	5	13	3	2.6	1.549	16 (76)	2.56	18 (86)	0.75

		(55)	(45)		(0.647- 4.215)	(24)	(62)	(14)	(0.592- 11.405)	(0.495- 4.847)		(0.622- 10.535)		(0.103- 5.411)
	DU (-)	41	23	0.26	1.00 (ref.)	12	17	3	1.00 (ref.)	1.00 (ref.)	20 (62)	1.00 (ref.)	29 (91)	1.00 (ref.)
		(64)	(36)			(38)	(53)	(9)						
	DU (+)	5	7	0.26	2.495	1	3	2	2.117	2.828	5 (83)	3.000	4 (67)	0.206
		(42)	(58)			(17)	(50)	(33)						
ANGPT2	rs3739390	C	G	$P^a$	OR  (95%CI)	CC	CG	GG	OR (95%CI) [CC/CG]	CG+GG	OR [CC/CG+ GG]  (95%CI)	N/A		
													ILD (-)	14
			(88)	(12)	(88)	(12)	(0)							
		ILD (+)	56	8	0.67	1.032	28	4	0	1.166 (0.109-12.471)	4 (12)		1.166	
		(88)	(12)	(0.196- 5.418)		(88)	(12)	(0)	(0.109- 12.471)					

	ACA	34	2	0.17	17	1	0	1.00 (ref.)	1 (4)	1.00 (ref.)		
		(94)	(6)			(95)	(5)					(0)
	Scl-70	36	8		3.777	18	4	0	3.777 (0.355-40.197)	4 (18)		3.777
		(82)	(18)		(0.729-19.561)	(82)	(18)	(0)				(0.355-40.197)

The OR and their 95% CIs were estimated by logistic regression models.

Between OR and (95% CIs) the information is added which category (group) is an outcome for the analysis according to either dominant or recessive model

<sup>a</sup> *P* value for allele frequencies (maximum Likelihood chi-squared test)

Abbreviations: ACA, anti-centromere antibodies; DUs, digital ulcers; ILD – interstitial lung disease; N/A, not applicable; OR, odds ratio; Scl-70, anti-scleroderma-70 antibodies; SNPs, single nucleotide polymorphisms; SSc, systemic sclerosis; 95%CI, 95% confidence intervals;

Table S4. Irrelevant associations between rs2442598 of ANGPT2 gene and risk of selected clinical variables among systemic sclerosis patients.

SNPs	Clinical variable	Allele frequency, n (%)					Genotype frequency, n (%)					
		T	C	G	<i>P</i> <sup>a</sup>	OR (95%CI)	CT	CC	GG	OR [CT/CC] (95%CI)	OR [CT/GG] (95%CI)	
ANGPT2 rs2442598	lcSSc	10	50	4	0.39	1.00 (ref.)	10	20	2	1.00 (ref.)	N/A	
		(16)	(78)	(6)			(31)	(63)	(6)			
	dcSSc	3	13	0		0.866 (0.203-3.699) <sup>b</sup>	3	5	0	0.833 (0.155-4.455)		
		(19)	(81)	(0)			(37.5)	(62.5)	(0)			
	DU <sub>s</sub> (-)	11	51	2	0.25	1.00 (ref.)	11	20	1	1.00 (ref.)	1.00 (ref.)	
		(17)	(80)	(3)			(34.5)	(62.5)	(3)			
	DU <sub>s</sub> (+)	2	8	2		0.862 (0.156-4.770) <sup>b</sup>	2	3	1	0.825 (0.110-6.131)		2.345 (0.412-13.344)
		(16.5)	(67)	(16.5)			2.345 (0.611-8.994) <sup>c</sup>	(33)	(50)			
	ILD (-)	2	14	0	0.56	1.00 (ref.)	2	6	0	1.00 (ref.)	N/A	
		(13)	(88)	(0)			(25)	(75)	(0)			

	ILD (+)	11	51	2	0.25	0.560 (0.110-2.852) <sup>b</sup>	11	20	1	0.538 (0.089-3.250)	N/A
		(17)	(80)	(3)			(34)	(63)	(3)		
	ACA	6	28	2		1.00 (ref.)	6	11	1	1.00 (ref.)	
		(17)	(78)	(5)			(33)	(61)	(6)		
	Scl-70	6	38	0		1.357 (0.387-4.748) <sup>b</sup>	6	16	0	1.454 (0.353-5.979)	
		(14)	(86)	(0)			(27)	(73)	(0)		

The OR and their 95% CIs were estimated by logistic regression models.

<sup>a</sup> *P* value for allele frequencies (maximum Likelihood chi-squared test)

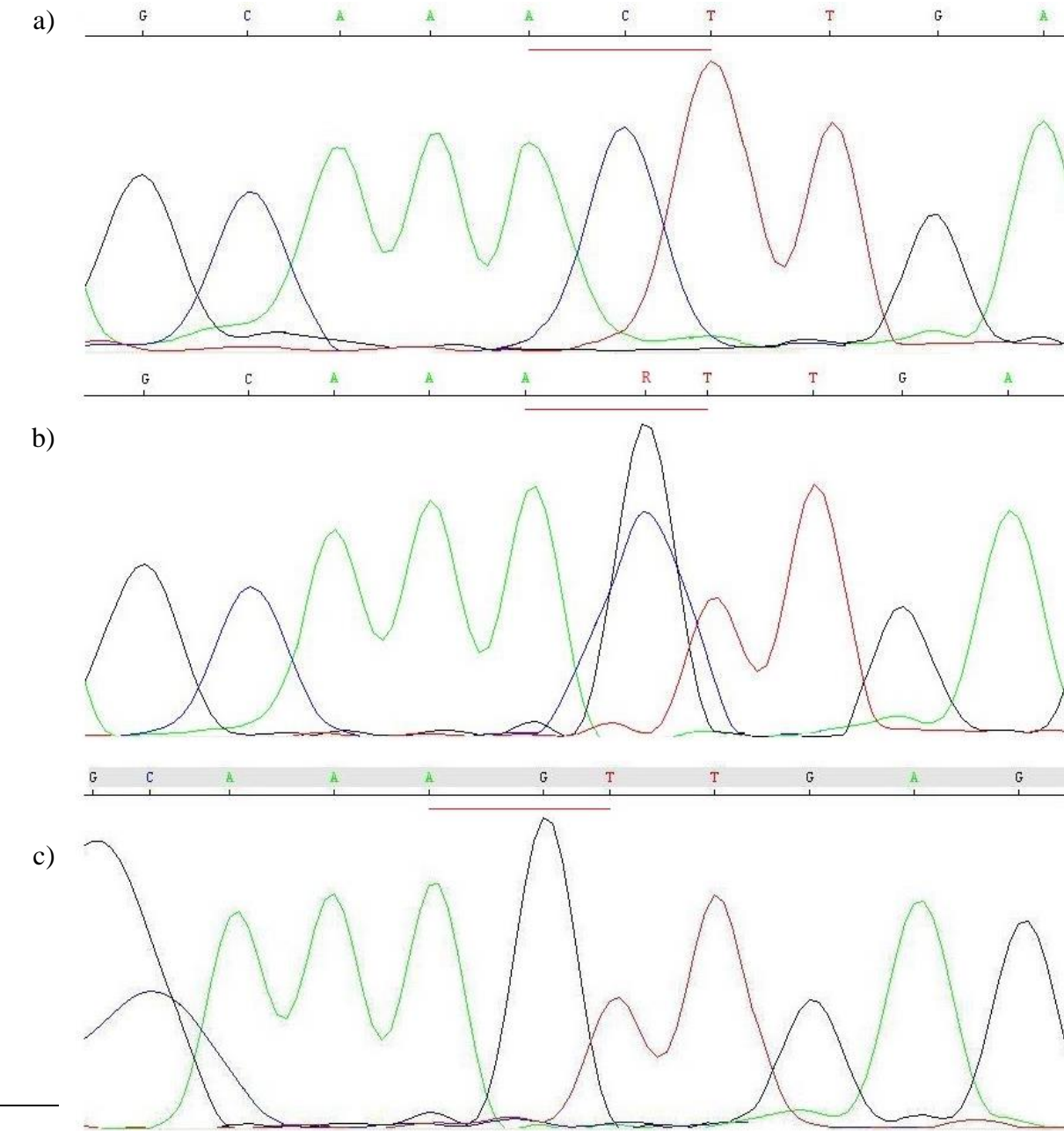
<sup>b</sup> for alleles T and C

<sup>c</sup> for alleles T and G

Abbreviations: ACA, anti-centromere antibodies; dcSSc, diffuse cutaneous systemic sclerosis; DUs, digital ulcers; ILD – interstitial lung disease; lcSSc, limited cutaneous systemic sclerosis; N/A, not applicable; OR, odds ratio; Scl-70, anti-scleroderma-70 antibodies; SNPs, single nucleotide polymorphisms; SSc, systemic sclerosis; 95%CI, 95% confidence intervals;

Figure S1. Electropherograms obtained by automated Sanger DNA sequencing. A) Sequencing of the ANGPT2 gene (rs3739390), the sequences referred to R strand; a) CC homozygote, b) CG heterozygote, and c) GG homozygote; B) Sequencing of the ANGPT2 gene (rs2442598), the sequences referred to R strand; a) CC homozygote, b) CT heterozygote, and c) TT homozygote

A.





B.

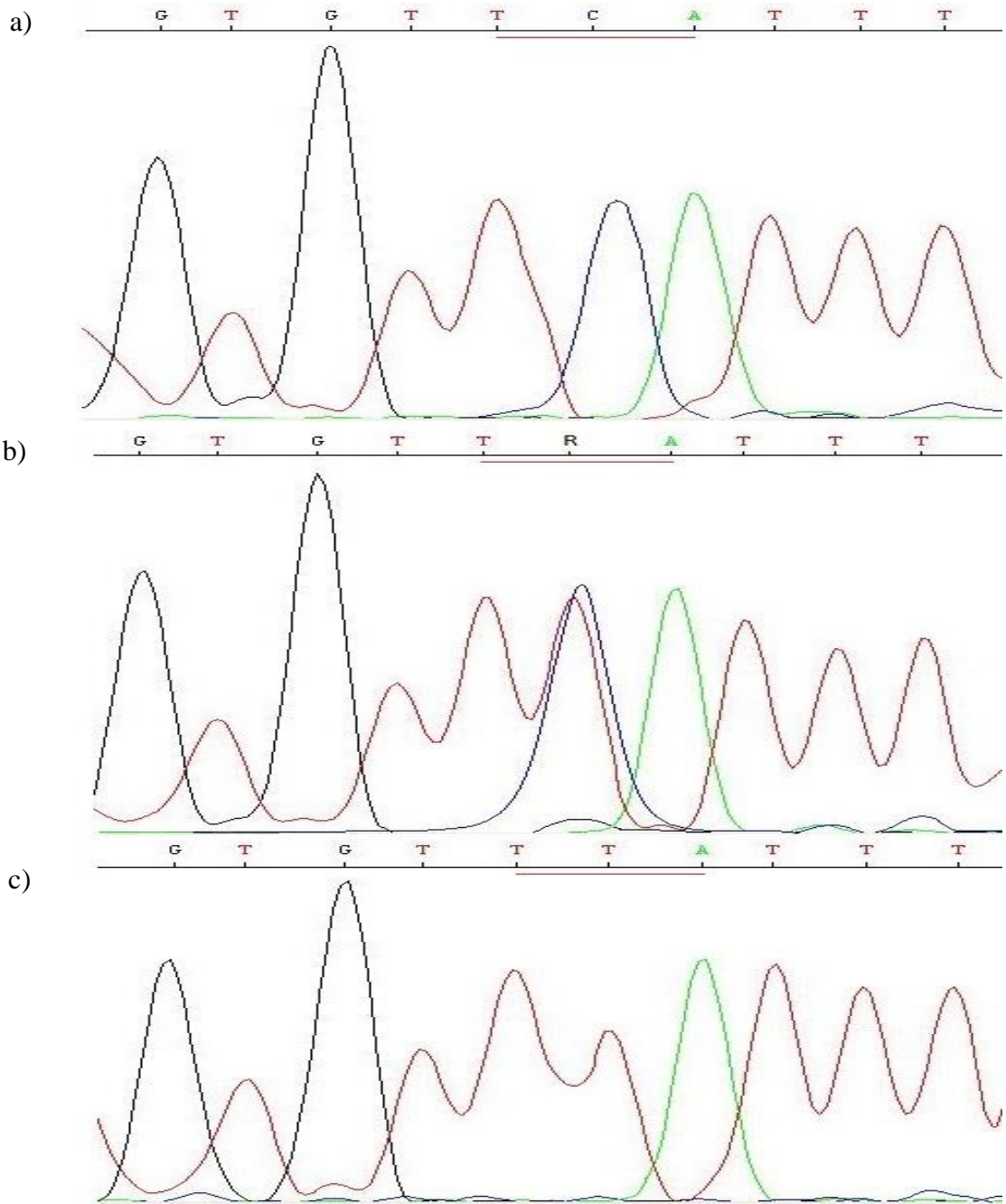


Figure S2. Allelic distribution for rs2507800 of Angiopoietin 1 gene (ANGPT1) in the studied population of systemic sclerosis patients and healthy controls.

