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# Angiotensin II Type 1 receptor (AGTR1) gene polymorphisms are associated with vascular manifestations in patients with systemic sclerosis (SSc)

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## Abstract

**Introduction:** Systemic sclerosis (SSc) shows variable clinical expression in different ethnic groups; vascular abnormalities are a prominent feature of this disease and its clinical expression may be influenced by genetic factors.

**Patients and methods:** Herein, we describe 15 polymorphisms of the renin-angiotensin-aldosterone pathway in 170 Mexican admixed SSc patients (defined as patients with Mexican ancestry for at least 3 generations) and 199 healthy controls. We determined the presence of angiotensin II Type 1 receptor (AGTR1), angiotensin converting enzyme (ACE) and Endothelin 1 single nucleotide polymorphisms (SNPs) using 5' exonuclease TaqMan genotyping assays on a 7900HT real-time fast polymerase chain reaction (PCR) system.

**Results:** These polymorphisms had a similar distribution between SSc patients and controls, but we found that the AGTR1 G-680T (rs275652) ($p = 0.02$; OR 3.5; 95%CI 1.2–10.4) and AGTR1 A-119G (rs275653) ($p = 0.008$; OR 4.2; 95% CI 1.5–12.1) polymorphisms were associated with severe vascular involvement in our SSc patients.

**Conclusions:** This is the first report of the association of these polymorphisms with vasculopathy in Mexican admixed SSc patients. Our findings suggested that the angiotensin II Type 1 receptor genotype may influence the clinical expression of vasculopathy in these patients. Functional analyses should follow.

## Keywords

Angiotensin, angiotensin II Type 1 receptor, finger ulcers, fingertip ulcers, Hispanic patients, polymorphisms, renin-angiotensin-aldosterone system, scleroderma, vasculopathy

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## Introduction

Systemic Sclerosis (SSc) is a systemic autoimmune connective tissue disorder characterized by inflammation, fibrosis and obliterative vasculopathy.[1] There are two clinical subsets of SSc described, based on the extent of cutaneous involvement: diffuse (dc) and limited cutaneous (lc) SSc.[2] These subtypes exhibit different profiles of autoantibodies, which also vary among populations.[3–10]

We have described that the prevalence of autoantibodies in Mexican SSc patients is different from that of other populations; that there is a higher prevalence of anti-topoisomerase I, anti-PM-Scl and anti-Ku antibodies and that there is a lower prevalence of anti-RNA polymerase III antibodies than in other populations.[10] These phenotypic variations are influenced by genetic background. For instance, the variability in the major histocompatibility complex (MHC) genes influences the persons' susceptibility to develop SSc and disease expression in different ethnic groups.[11–17]

Vascular involvement is a primary event in SSc pathogenesis. Studies of angiotensin converting enzyme (ACE) and endothelial nitric oxide synthase (eNOS) polymorphisms suggest that specific alleles are associated with scleroderma[18]; however, there is no information about the role of these and other polymorphisms being involved the renin-angiotensin-aldosterone system (RAAS) in Mexican admixed SSc patients (defined as patients with Mexican ancestry for at least 3 generations). This study will identify the influence of these polymorphisms in the presence and severity of internal organ damage in this patient population.

## Patients and methods

### Subjects

We evaluated 15 functional single nucleotide polymorphisms (SNPs) in angiotensin-converting enzyme (ACE), angiotensin II Type 1 receptor (AGTR1) and endothelin 1 genes (Supplementary Table 1), in a group of 170 Mexican admixed SSc patients (77 dcSSc and 93 lcSSc) without overlap syndromes, and in 199 ethnically matched healthy controls.

Patients fulfilled the 2013 classification criteria for SSc[19] or LeRoy-Medsger criteria for early SSc.[20] All patients were evaluated by one rheumatologist, who was responsible for the scleroderma cohort, between July 2007 and July 2010 at the *Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán* (INCMNSZ), a referral center in Mexico City, Mexico. Patients were classified as dcSSc or lcSSc (skin involvement above the elbows or knees, or including chest or abdomen, at any time during the illness for the diffuse disease; and distal to the elbows or knees for the limited disease).[10] Organ involvement attributable to SSc was also determined using previously published definitions.[21] The severity of each organ manifestation was determined according to the Medsger severity scale, at the time of the inclusion of the patient to this study; scores of 3–4 for each organ system were considered as severe involvement.[21]

*Ethics statement.* Local Institutional Review Boards (IRBs) approved the study. All subjects provided written informed consent for collection of clinical data, serum and DNA samples. All patients were at least 18 years old. This study was carried out in accordance with the World Medical Association Declaration of Helsinki.

### SSc-specific autoantibodies

Peripheral venous blood was obtained to isolate serum (frozen at −70°C until processing) and we tested it for SSc-associated antibodies, according to the manufacturers' recommendations: the antinuclear IgG antibodies were detected by indirect immunofluorescence using a Hep-2 cell substrate (The Binding Site, Birmingham, UK)[10] ; the IgG isotype anti-topoisomerase-I, anticentromere B, anti-U1 RNP (The Binding Site, UK) and anti-RNA polymerase III (INOVA Diagnostics, San Diego, CA, USA) were detected by immunoenzymatic assay (EIA). For the anticentromere A, anti-U3 RNP, anti-U11/U12 RNP, anti-PM-Scl, anti-Th/To and anti-Ku antibodies, a commercial Western blot was performed using Hep-2 whole cell extract and recombinant centromere A, U3 RNP, U11/U12 RNP, PM-Scl, Th/To and Ku proteins (Euroimmun, Lübeck, Germany).

### SNP analysis

Genomic DNA was isolated from peripheral blood mononuclear cells (PBMCs). SNPs were genotyped using 5' exonuclease TaqMan genotyping assays on a 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). Each genotype was defined with the allelic discrimination software (7300 System SDS Software by Applied Biosystems, USA).

### Statistical analysis

Demographic and clinical variables were analyzed with SPSS v15. We tested for the Hardy-Weinberg equilibrium for all genotypic combinations of each SNP, in patients and controls. We evaluated the differences in the distribution of the allelic and genotypic frequencies using the Mantel-Haenszel Chi-square test (EPIINFO v6.04b). We used a significance level of $p < 0.05$, with Bonferroni correction. Odds ratios (OR) with 95% CI were estimated. We performed logistic regression analyses in the variables that showed $p$ values of $< 0.1$ for SNP association, to adjust for confounding variables.

**Table 1.** Clinical and serological characteristics of patients with SSc.[a]

| | Total *n* = 170 (%) | dcSSc *n* = 77 (%) | lcSSc *n* = 93 (%) | *p* value |
|---|---|---|---|---|
| Female gender | 157 (92) | 67 (87) | 90 (97) | 0.01 |
| Age (mean) | 45.1 ± 14.8 | 42.5 ± 13.9 | 47.7 ± 15.1 | 0.02 |
| Time of evolution (mean) | 11 ± 9.7 | 8 ± 6 | 13 ± 1.1 | 0.001 |
| Vascular | 155 (91) | 73 (95) | 82 (88) | 0.37 |
| Severe vascular[b] | 79 (46) | 41 (53) | 38 (41) | 0.19 |
| Tendons/joints | 116 (68) | 63 (82) | 53 (57) | 0.001 |
| Severe[b] tendons/joints | 29 (17) | 18 (23) | 11 (12) | 0.04 |
| Muscular | 26 (15) | 13 (17) | 13 (17) | 0.6 |
| Severe[b] muscular | 2 (1) | 1 (1) | 1 (1) | 0.8 |
| Gastrointestinal | 118 (69) | 61 (79) | 57 (61) | 0.01 |
| Severe[b] gastrointestinal | 7 (4) | 5 (6.5) | 2 (2) | 0.15 |
| ILD | 77 (45) | 44 (57) | 33 (35) | 0.005 |
| Severe[b] ILD | 12 (7) | 8 (10) | 4 (4) | 0.11 |
| PAH | 42 (25) | 23 (30) | 19 (20) | 0.13 |
| Severe[b] PAH | 6 (3.5) | 3 (4) | 3 (3) | 0.8 |
| Heart | 23 (13) | 12 (16) | 11 (12) | 0.29 |
| Severe[b] heart | 3 (2) | 1 (1) | 2 (2) | 0.9 |
| Renal | 2 (1) | 1 (1) | 1 (1) | 0.6 |
| Severe[b] renal | 2 (1) | 1 (1) | 1 (1) | 0.2 |
| Comorbidities | Total *n* = 170 (%) | dcSSc *n* = 77 (%) | lcSSc *n* = 93 (%) | *p* value |
| Diabetes mellitus | 9 (5.2) | 4 (5) | 5 (5) | 0.9 |
| Systemic arterial hypertension | 19 (11) | 6 (8) | 13 (14) | 0.19 |
| Dyslipidemia | 19 (11) | 7 (9) | 12 (13) | 0.41 |
| Current tobacco use | 12 (7) | 6 (8) | 6 (6.5) | 0.7 |
| Past tobacco use | 37 (22) | 23 (30) | 14 (15) | 0.02 |
| Coronary artery disease | 8 (5) | 3 (4) | 5 (5) | 0.64 |
| Cerebral vascular disease | 1 (0.6) | 0 (0) | 1 (1) | 0.3 |
| Anti-phospholipid antibody syndrome | 3 (2) | 0 (0) | 3 (3.2) | 0.11 |
| Anti-phospholipid antibody positive | 36 (21) | 15 (19.5) | 21 (23) | 0.86 |
| SSc-associated autoantibodies | Total *n* = 170 (%) | dcSSc *n* = 77 (%) | lcSSc *n* = 93 (%) | Total *n* = 170 (%) |
| Anti-centromere | 43 (25) | 15 (19.5) | 28 (30) | 0.09 |
| Anti-topoisomerase I | 38 (22) | 32 (42) | 6 (6.5) | 0.0009 |
| Anti-U1-RNP | 27 (16) | 5 (6.5) | 22 (24) | 0.002 |
| Anti-PM-Scl | 10 (6) | 5 (6.5) | 5 (5) | 0.8 |
| Anti-RNA-Pol III | 1 (0.6) | 0 (0) | 1 (1) | 0.9 |
| Anti-Ku | 11 (6.5) | 4 (5) | 7 (7.5) | 0.5 |
| Only positive ANA | 38 (22) | 15 (19.5) | 23 (25) | 0.4 |
| No detectable autoantibody | 2 (1) | 1 (1) | 1 (1) | 0.9 |

[a]Data are expressed as means ± SD, means and percentage or *n* and percentage.
[b]Items marked as 'severe' are those with Medsger Severity Scale scores of 3 or 4.
ANA: anti-nuclear antibodies; dcSSc: diffuse cutaneous systemic sclerosis; ILD: interstitial lung disease; Anti-Ku: anti-Ku antibodies; lcSSc: limited cutaneous systemic sclerosis; PAH: pulmonary arterial hypertension; Anti-PM-Scl: anti-PM-Scl antibodies; Anti-RNA-Pol III: anti-RNA-Pol III antibodies; Anti-UI-RNP: anti-UI-RNP antibodies; SSc: systemic sclerosis.

## Results

Demographic and clinical characteristics are shown in Table 1. There were more male patients in the dcSSc (13%) group than in the lcSSc group (3%; $p = 0.01$). Tendon and joint involvement ($p = 0.001$), gastrointestinal damage ($p = 0.01$) and interstitial lung disease ($p = 0.005$) were more frequent in dcSSc than lcSSc patients.

AGTR1, ACE and endothelin 1 polymorphisms had similar distributions between SSc patients and controls

**Table 2.** Association of the AGRT1 G-680 T and AGRT1 A-119 G polymorphisms with severe vascular damage in SSc patients.

| Gene | Severe vascular involvement ($n$ = 79) | No severe vascular involvement ($n$ = 80) | pC value, OR, 95% CI | $p$[a] value after logistic regression, OR, 95% CI |
|---|---|---|---|---|
| AGTR1 G-680T (rs275652) | | | | |
| Genotypes | $n$ (g.f.) | | | |
| GG | 0 | 2 (0.025) | 0.49 | |
| GT | 17 (0.220) | 4 (0.050) | 0.006, OR 5.2 (1.6–16.2) | 0.02, OR 3.5, (1.2–10.4) |
| TT | 62 (0.780) | 74 (0.925) | 0.02, OR 0.3 (0.1–0.8) | NS |
| Alleles | $n$ (a.f.) | | | |
| G | 17 (0.108) | 8 (0.050) | 0.02, OR 3.3 (1.2–9.1) | |
| T | 141 (0.892) | 152 (0.950) | 0.49, OR 2.01 (1.7–2.3) | |
| AGTR1 A-119G (rs275653) | | | | |
| Genotypes | $n$ (g.f.) | | | |
| GG | 0 | 2 (0.025) | 0.49 | |
| AG | 19 (0.241) | 4 (0.050) | 0.001, OR 6 (1.9–18.6) | 0.008, OR 4.2 (1.5–12.1) |
| AA | 60 (0.759) | 74 (0.925) | 0.008, OR 0.26 (0.09–0.7) | |
| Alleles | $n$ (a.f.) | | | |
| G | 19 (0.120) | 8 (0.050) | 0.008, OR 3.9 (1.4–10.4) | |
| A | 139 (0.880) | 152 (0.950) | 0.04, OR 0.39 (0.15–0.97) | |

[a]Associations in this column were tested using logistic regression adjusting for age, gender, arterial hypertension, dyslipidemia and Type 2 diabetes mellitus.
AGTR1: angiotensin II Type I receptor; a.f.: allele frequency, g.f.: genotype frequency; pC: corrected $p$ value (by Bonferroni).

(Supplementary Table 2), and between diffuse and limited SSc patients (Supplementary Table 3); however, the analysis of these polymorphisms by internal organ involvement revealed that there were some differences in AGTR1 SNPs.

### *AGTR1-680 and -119 SNPs were associated with severe vascular involvement in SSc patients*

Table 2 and Supplementary Table 4 show a significant increase in the genotype frequency of the heterozygous G/T genotype of the AGTR1 G-680T polymorphism (rs275652) in the group of SSc patients with severe vascular involvement (presence ever of digital tip ulcers and/or digital gangrene, according to the Medsger Severity Scale[21]; g.f. = 0.220), when compared with SSc patients without severe vascular involvement (g.f. = 0.050; pC = 0.006; OR = 5.2 and 95%CI = 1.6–16.2). Accordingly, we observed a significant association of the G allele with severe vascular involvement (pC = 0.02; OR = 3.3 and 95% CI = 1.2–9.1).

The analysis of the AGTR1 A-119G polymorphism (rs275653) showed (Table 2) a higher frequency of the A/G heterozygous genotype in the group of SSc patients with severe vascular involvement (g.f. = 0.241 versus g.f. = 0.050 in the SSc patients without severe vascular damage, pC = 0.001, OR = 6.0 and 95%CI = 2.0–18.6). In concordance, we detected a significant increase in the frequency of the G allele in the severe vascular involvement group (pC = 0.008; OR = 3.9 and 95%CI = 1.4–10.4).

Importantly, we confirmed these associations by logistic regression analysis after adjustment for age, gender, arterial hypertension, dyslipidemia and Type II diabetes mellitus (Table 2).

## Discussion

Here we studied the prevalence of RAAS polymorphisms and described, for the first time, their associations with organ involvement in Mexican admixed SSc patients. The most significant findings confirmed by logistic regression analysis were the associations of the heterozygous genotypes GT of the SNP AGTR1-680 (rs275652) and AG of the SNP AGTR1-119 (rs275653) with severe vascular damage in SSc patients. These polymorphisms are located in the promoter region of the AGTR1 gene on chromosome 3q21-25. Genetic variations of this gene have been associated with the susceptibility to different diseases.[23–26]

The AGTR1 gene encodes the Type 1 angiotensin II receptor, which is thought to mediate the major cardiovascular effects of angiotensin II (induction of aldosterone synthesis, increase of water and salt resorption, and potassium excretion in the kidney and increase in blood pressure). Multiple alternatively spliced transcript variants have been reported for this gene.[27]

Several mechanisms are involved in vascular injury in SSc, including the effect of auto-antibodies against endothelial cells, autoreactive T cells, impaired vasculogenesis and endothelial dysfunction that promotes tissue hypoxia and fibrosis.[28]

It is well known that alterations in the signaling of the renin-angiotensin-aldosterone system also contribute to vascular injury, inflammation and remodeling in diseases like hypertension, atherosclerosis and cardiac failure. The chronic inflammatory state in the vascular wall contributes to the endothelial impairment that results in increased inflammatory infiltrate and tissue injury.[27] Administration of losartan, an angiotensin II receptor 1 (ATR1) antagonist, inhibits Ang II-induced NF-κB activation and vascular cell adhesion molecule 1 (VCAM-1) accumulation,[29] suppresses the function of toll-like receptors (TLR)[30] 2 and 4 (TLR2 and TLR4) and the production of inflammatory mediators, such as C-reactive protein (CRP) and interleukin 6 (IL-6).[31] In this regard, it is well known that SSc patients show increased levels of VCAM-1 in vascular tissue, and elevated serum CRP and IL-6 levels.[28]

Recent reports demonstrate that there is a significant decrease of Sma mothers against decapentaplegic (SMAD) 2/3 (Smad2/3) molecules, collagen concentration and alpha smooth muscle actin (α-SMA) expression in an experimental mouse SSc model treated with irbesartan.[32] It is also known that exposure to the transforming growth factor beta (TGF-B) of lung fibroblasts from patients with lung fibrosis strongly induces the expression of AGTR1[33]; hence, polymorphisms in this pathway may modulate the expression of inflammatory and vascular abnormalities.[34] Moreover, variants of polymorphisms that are located in the AGTR1 gene promoter region could modulate the expression of AGTR1 mRNA levels, or influence alternative splicing in target cells in SSc patients. The overexpression of AGTR1 mRNA could promote increased vasoconstrictive effects of angiotensin II, and possibly contribute to the development of vascular hypoxia, injury and fibrosis in SSc patients. It is interesting to note that the AGTR1-680 (rs275652) and AGTR1-119 (rs275653) polymorphisms were not associated with the SSc itself, but with the presence of severe vascular disease (patients with the presence or history of ischemic digital tip ulcers and/or necrosis); hence, it seems that the susceptibility variants of these polymorphisms confer additional risk for developing a severe form of vasculopathy in SSc patients.

Our study has some limitations, including its relatively small sample size, restricted by the study's focus on SSc patients from Mexican admixed ancestry, and the lack of functional assays to determine the effects of the AGTR1 gene variation in severe vascular involvement in SSc patients and healthy controls; however, one of this study's strengths included the analysis of the influence of AGTR1 promoter polymorphisms in an adequately characterized cohort of Mexican SSc patients and healthy controls.

In summary, our results suggest that the polymorphisms rs275652 and rs275653, located in the promoter region of the AGTR1 gene, may contribute to SSc patients' susceptibility to severe vascular involvement.

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## Declaration of conflicting interests

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## References

1. Mayes M and Reveille J. Epidemiology, demographics and genetics. In: *Systemic sclerosis*. 2nd ed. Philadelphia, PA, USA: Lippincott Williams and Wilkins, 2004, pp. 1–15.
2. Medsger TA Jr. Classification, prognosis. In: *Systemic sclerosis*. 2nd ed. Lippincott Williams and Wilkins, 2004, pp. 17–28.
3. Hu PQ, Fertig N, Medsger TA, et al. Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis. *Arthritis Rheum* 2003; 48: 1363–1373.
4. Okano Y, Steen V and Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. *Ann Intern Med* 1996; 119: 1005–1013.
5. Aggarwal R, Lucas M, Fertig N, et al. Anti-U3 RNP autoantibodies in systemic sclerosis. *Arthritis Rheum* 2009; 60: 1112–1118.
6. Mitri GM, Lucas M, Fertig N, et al. A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. *Arthritis Rheum* 2003; 48: 203–209.
7. Williams J, Lucas M, Fertig N, et al. Anti-Ku antibody in patients with systemic sclerosis: Comparison of clinical features associated with anti-U1 RNP, anti-U3 RNP and anti-PM-Scl antibodies. *Arthritis Rheum* 2005; 52: S590–591.
8. Fertig N, Domsic RT, Rodriguez-Reyna T, et al. Anti-U11/U12 RNP antibodies in systemic sclerosis: A new serologic marker associated with pulmonary fibrosis. *Arthritis Rheum* 2009; 61: 958–965.
9. Mayes MD, Lacey JV Jr, Beebe-Dimmer J, et al. Prevalence, incidence, survival and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003; 48: 2246–2255.
10. Rodriguez-Reyna TS, Hinojosa-Azaola A, Martínez-Reyes C, et al. Distinctive autoantibody profile in Mexican mestizo systemic sclerosis patients. *Autoimmunity* 2011; 44: 576–584.
11. Gorlova O, Martin JE, Rueda B, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. *PLoS Genet* 2011; 7:e1002178. Doi: 10.1371/journal.pgen.1002178.

12. Arnett FC, Gourh P, Shete S, et al. Major histocompatibility complex (MHC) Class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: Analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann Rheum Dis* 2010; 69: 822–827.
13. Gladman DD, Kung TN, Slannis F, et al. HLA markers for susceptibility and expression in scleroderma. *J Rheumatol* 2005; 32: 1481–1487.
14. Kuwana M, Inoko H, Kameda H, et al. Association of human leukocyte antigen class II genes with autoantibody profiles, but not with disease susceptibility in Japanese patients with systemic sclerosis. *Intern Med* 1999; 38: 336–344.
15. Reveille JD, Owerbach D, Goldstein R, et al. Association of polar aminoacids at position 26 of the HLA-DQB1 first domain with the anti-centromere autoantibody response in systemic sclerosis (scleroderma). *J Clin Invest* 1992; 89: 1208–1213.
16. Reveille JD, Durban E, MacLeod-St Clair MJ, et al. Association of aminoacid sequences in the HLA-DQB1 first domain with anti-topoisomerase I autoantibody response in scleroderma (progressive systemic sclerosis). *J Clin Invest* 1992; 90: 973–980.
17. Rodriguez-Reyna TS, Mercado-Velázquez P, Yu N, et al. HLA Class I and II blocks are associated with susceptibility, clinical subtypes and autoantibodies in Mexican systemic sclerosis (SSc) patients. *PloS One* 2015; 10: e0126727.
18. Fatini C, Gensini F, Sticchi E, et al. High prevalence of polymorphisms of angiotensin-converting enzyme (I/D) and endothelial nitric oxide synthase (Glu298Asp) in patients with systemic sclerosis. *Am J Med* 2002; 112: 540–544.
19. Van den Hoogen F, Khanna D, Fransen J, et al. 2013 Classification criteria for systemic sclerosis. An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737–2747.
20. LeRoy EC and Medsger TA Jr. Criteria for the classification of early systemic sclerosis. *J Rheumatol* 2001; 28: 1573–1576.
21. Medsger TA Jr, Bombardieri S, Czirjak L, et al. Assessment of disease severity and prognosis. *Clin Exp Rheumatol* 2003; 21: S60–64.
22. Cutolo M, Sulli A and Smith V. Assessing microvascular changes in systemic sclerosis diagnosis and management. *Nat Rev Rheumatol* 2010; 6: 578–587.
23. Mishra A, Srivastava A, Mittal T, et al. Genetic predisposition to left ventricular dysfunction: A multigenic and multianalytical approach. *Gene* 2014; 546: 309–317.
24. Anbazhagan K, Sampathkumar K, Ramakrishnan M, et al. Analysis of polymorphism in renin angiotensin system and other related genes in South Indian chronic kidney disease patients. *Clin Chim Acta* 2009; 406: 108–112.
25. Mohamed S, Schaa K, Cooper ME, et al. Genetic contributions to the development of retinopathy of prematurity. *Pediatr Res* 2009; 65: 193–197.
26. Pop D, Zdrenghea D, Procociuc LM, et al. Gene polymorphism of angiotensin-converting enzyme and angiotensin II Type 1 receptor in patients with congestive heart failure. *Rom J Intern Med* 2007; 45: 349–354.
27. Ruiz-Ortega M, Ruperez M, Esteban V, et al. Molecular mechanisms of angiotensin II-induced vascular injury. *Curr Hypertens Rep* 2003; 5: 73–79.
28. Asano Y and Sato S. Vasculopathy in scleroderma. *Semin Immunopathol* 2015; 37: 489–500.
29. Tummala PE, Chen X-L, Sundell CL, et al. Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: A potential link between the renin-angiotensin system and atherosclerosis. *Circulation* 1999; 100: 1223–1229.
30. Mason RP. Optimal therapeutic strategy for treating patients with hypertension and atherosclerosis: Focus on olmesartan medoxomil. *Vasc Health Risk Managem* 2011; 7: 405–416.
31. Sola S, Mir MQS, Cheema FA, et al. Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: Results of the irbesartan and lipoic acid in endothelial dysfunction (ISLAND) study. *Circulation* 2005; 111: 343–348.
32. Marut W, Kavian N, Servettaz A, et al. Amelioration of systemic fibrosis in mice by angiotensin II receptor blockade. *Arthritis Rheum* 2013; 65: 1367–1377.
33. Renzoni EA, Abraham DJ, Howat S, et al. Gene expression profiling reveals novel TGFbeta targets in adult lung fibroblasts. *Respir Res* 2004; 5: 24.
34. Benigni A, Orisio S, Noris M, et al. Variations of the angiotensin II Type 1 receptor gene are associated with extreme human longevity *Age* 2013; 35: 993–1005.