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# Association of Urotensin II Gene Polymorphism with Disease Pattern in Systemic Sclerosis Patients

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## Abstract

**Background:** Urotensin II (U-II) and its receptor, UT, are widely expressed throughout the body. It is known to be the most potent endogenous vasoconstrictor discovered to date. Its physiological mechanisms are similar in some ways to Endothelin 1 (ET-1).

**Objective:** To investigate the possible association of the polymorphism of UTS2 gene (Thr21Met and Ser89Asn) with the susceptibility to SSc and pattern of disease manifestations in Egyptian patients.

**Results:** We found that the Thr21Met MM genotypes of the UTS2 gene were significantly increased in SSc patients (20%) compared to the control subjects (5%) (p=0.013) There was a significant difference in the MM and NN genotypes in diffuse SSc patients (35.7%, 67.9%) compared to limited SSc patients (6.2%, 25%) (p=0.004, 0.001) .The frequency of the N allele of the UT89 gene polymorphism was significantly higher in the SSc patients (58.3%) than in the control subjects (36.7%) (p=0.001). Significant associations were found between Thr21Met MM and Ser89Asn NN genotypes with pitting scars, digital ischemia, pulmonary hypertension, gastrointestinal manifestations and Anti Scleroderma-70 Antibody. Significant associations were found between Thr21Met M allele with pitting scars, digital ischemia and Anti Scleroderma-70 Antibody, while Ser89Asn N allele showed also in addition a significant association with Raynaud's phenomenon and renal manifestations.

**Conclusion:** The results suggest that UTS2 Thr21Met and Ser89Asn genetic polymorphisms may be important risk factors in the development of SSc susceptibility in the Egyptian population, and an indicator of many disease manifestations such as pulmonary hypertension, severe skin and lung involvement in patients with SSc.

**Keywords:** Systemic sclerosis; Urotensin-II; UTS2Thr21Met genetic polymorphisms; Ser89Asn genetic polymorphisms

## Abbreviations

SSc: Systemic Sclerosis; U-II: Urotensin-II

## Introduction

Scleroderma, or systemic sclerosis (SSc), is a multifactorial, chronic fibrotic collagen tissue disorder that is mainly based on 3 pathogenetic features: accumulation of extracellular matrix, vasculopathy of the small vessels, and autoimmunity. Clinical and experimental data show that the pathogenesis of SSc is multifactorial, involving both genetic and environmental Factors [1,2]. The most prominent manifestations of SSc are abnormalities of the circulation and involvement of multiple organ systems, including the musculoskeletal, renal, pulmonary, cardiac, and gastrointestinal systems, with fibrotic and/or vascular Complications [3]. Although cytokines and immune mediators play a major role in the initiation and progression of disease, the importance of the genetic component in SSc pathogenesis has not been fully defined [4].

Endothelin-1 (ET-1), known to be one of the leading peptides, has a pivotal role from the early to late phases of this illness [5]. ET-1 and ET-1 receptor binding proteins are elevated in SSc serum and tissue samples. This is prominent especially if patients have vascular, lung, skin, and renal involvement [6]. We know that ET-1 might coordinate inflammation, vasculopathy, tissue regeneration, and fibrosis in SSc. Several treatment strategies based on targeting ET-1 showed unusual reduction in morbidity and mortality [7,8]. Urotensin-II (U-II) is a new peptide with various effects in different tissues of the human body. It is a potent vasoconstrictive agent with 50 times more effect on the arterial system and nearly ten times more effect on the venous system than ET-1. ET-1 always displays effects on the vasculature in the same direction as a vasoconstructant [9]. However, the effect of U-II alters depending on the diameter of the vessel and vascular bed. Both of these peptides are potent mitogenic agents, and they both have pro-inflammatory and pro-oxidative features, and, therefore, they both have roles in renal and cardiovascular diseases [10]. U-II seems to be one of the most important actors of Pulmonary Arterial Hypertension (PAH) pathogenesis [11]. Considering the frequent vascular findings of SSc like Reynaud's phenomenon, PAH, renal crisis, digital ulcers, and gangrene, it is possible for U-II to be associated with endothelial dysfunction, vasculopathy, and capillary disorders of this disease. Also,

some studies found out that patients with heart failure, hypertension, diabetes mellitus and renal dysfunction has higher U-II levels in contrast to the control group [8-10].

The gene for U-II (UTS2) is located in human chromosome 1p36-p32 [12]. According to the US National Center for Biotechnology Information (NCBI) database, over 60 single- nucleotide polymorphisms (SNP) have been noted in the human UTS2 gene. Three of these SNP show amino acid changes in the UTS2 gene sequence. High allelic frequencies in Japanese populations were documented for the Thr21Met and Ser89Asn polymorphisms [12,13]. Pehlivan et al., 2012 found that there is association between Thr21Met, but not Ser89Asn, in the UTS2 gene and SSc in a Turkish population [14].

This study aimed to explore the association between U-II gene polymorphisms (Thr21Met and Ser89Asn) and pattern of disease manifestations in SSc in an Egyptian population.

## Patients and Methods

This case control study was conducted in Faculty of Medicine, Cairo University in the period between June 2013 and December 2014. This study was approved by the Ethical Committee of Kasr Al Ainy Medical Hospital and was in accordance with the principle of Helsinki Declaration. A written informed consent was obtained from each participant.

### Study groups

One hundred and twenty subjects participated in the present study. They were classified into 2 main groups:

**Group I:** Sixty inpatient and/or outpatient Egyptian patients with the diagnosis of SSc (48 females/12 males) with a mean age of (42.75 ± 12.51) years, all patients were diagnosed according to the American College of Rheumatology European League Against Rheumatism Collaborative Initiative (ACR/EULAR) [15].

**Group II:** Sixty unrelated healthy controls (49 females/11 males) with a mean age of (43.00 ± 8.95) years, with similar demographic characteristics.

### Exclusion criteria

1.  Patients with skin thickening sparing the fingers or patients who have a scleroderma-like disorder that better explains their manifestations (e.g., nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fasciitis, scleredema diabeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy).
2.  Patients with hypertension, hyperlipidemia, diabetes mellitus, renal dysfunction, or additional chronic or other autoimmune diseases.

For each patient, we obtained detailed information regarding demographic characteristics; age at first diagnosis, SSc clinical manifestation and the following investigations will be done; complete blood picture and ESR, antinuclear antibodies, kidney functions and urine analysis, electrocardiography (ECG), echocardiography (ECHO), lung radiographs and pulmonary function. U-II gene polymorphisms

(Thr21Met and Ser89Asn) were detected by PCR (using the components of the mix produced by Biorion (Rheinhorststr. 18-67071 Ludwigshafen, Germany. Catalog number 101605) followed by restriction fragment length polymorphism analysis (RFLP).

### Statistical analysis

1.  Data were coded and entered using the statistical package SPSS version 21. Data were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables.
2.  Genotype and allele frequencies were compared between the disease and the control groups using Chi-square tests. Fisher's exact test was used instead when the expected frequency is less than 5. Haplotypes were constructed and compared between cases and control. Odds ratio (OR) with 95% confidence intervals (95%CI) was calculated.
3.  Association of SSc clinical manifestations with genotype was performed using binary logistic regression and incorporating gender, age at first diagnosis, and disease duration as covariates.
4.  The nonparametric Mann–Whitney U test was used to compare numeric variables between genotype groups and between cases and control. p value <0.05 was considered as statistically significant.

### Results

We investigated 60 SSc patients (12 males and 48 females); their mean age was 42.75 ± 12.51 years. While, in the control group (n=60), 11 of them were males and 49 were females, their mean age was 43 ± 8.95. The SSc patients were further classified according to LeRoy et al. [16] into 32 patients (53.3%) with limited Scleroderma and 28 patients (46.6%) with diffuse Scleroderma, their demographic features are shown in Table 1.

| | | Limited scleroderma (32) (Mean ± SD) | Diffuse scleroderma (28) (Mean ± SD) | p value |
|---|---|---|---|---|
| Sex | Male N (%) | 10 (31.2%) | 2 (7.1%) | 0.020 |
| | Female N (%) | 22 (68.8%) | 26 (92.9%) | |
| Age (years) | | 39.03 ± 13.31 | 47.00 ± 10.18 | 0.015 |
| Age of onset(years) | | 33.62 ± 13.15 | 43.14 ± 12.12 | 0.002 |
| Disease duration (years) | | 5.41 ± 3.54 | 3.86 ± 3.31 | 0.057 |

**Table 1:** Comparison between demographic data of limited and diffuse scleroderma patients.

Patients with diffuse Scleroderma showed significant higher rates of digital ischaemia (p=0.002), pitting scars (p<0.001), pulmonary hypertension (p=0.009), pulmonary manifestations (p=0.014), GIT manifestations in the form of dysphagia, reflux and dysmotility (p=0.021) and renal manifestations in the form of proteinuria (p=0.034) compared to those of limited subtype (Table 2).

|  | Limited scleroderma (32) N (%) | Diffuse scleroderma (28) N (%) | p value |
|---|---|---|---|
| Raynaud's Phenomenon | 25(78.1%) | 26(92.9%) | 0.155 |
| Pitting scars | 6(18.8%) | 22(78.6%) | <0.001 |
| Digital ischaemia | 10(31.2%) | 20(71.4%) | 0.002 |
| Arthritis | 8(25.0%) | 10(35.7%) | 0.366 |
| GIT manifestations | 11(34.4%) | 18(64.3%) | 0.021 |
| Pulmonary manifestations | 14(43.8%) | 21(75.0%) | 0.014 |
| Pulmonary Hypertension | 12(37.5%) | 20(71.4%) | 0.009 |
| Cardiac manifestations | 4(12.5%) | 8(28.6%) | 0.121 |
| Renal manifestations | 4(12.5%) | 10(35.7%) | 0.034 |
| Anti-Nuclear Antibodies | 31(96.9%) | 18(64.3%) | 0.001 |
| anti scl 70 antibodies | 11(34.4%) | 22(78.6%) | 0.001 |
| ACA | 24(75%) | 14(50%) | 0.045 |

**Table 2:** Comparison between clinical and immunological features of limited and diffuse scleroderma patients.

The frequencies of MM, T/M and TT genotypes of the UT 21 polymorphism in SSc patients were 20%, 80% and 0%, respectively in SSc patients. While, the frequencies of MM, T/M and TT genotypes of the UT 21 polymorphism in control group were 5%, 95% and 0 %, respectively. A significant difference was observed in the MM genotype in SSc patients (20%) compared to the control subjects (5%) (p=0.013; OR=4.750; 95%CI=1.266-17.819). While, no significant difference was detected between the M and T allele in both groups (Table 3).

| | | Control (n=60)(%) | SSc (n=60)(%) | p value | OR (95% CI) |
|---|---|---|---|---|---|
| UT21 Geno-type | M/M | 3 (5.0%) | 12 (20.0%) | 0.013 | 4.750 (1.266-17.819) |
| | T/M | 57 (95.0%) | 48 (80.0%) | | 0.211 (0.056-0.790) |
| | T/T | 0 (.0%) | 0 (0%) | | |
| | M | 63 (52.2%) | 72 (60%) | 0.242 | 1.357 (0.814-2.264) |
| | T | 57(47.8%) | 48 (40%) | | Reference |
| UT89 Geno-type | N/N | 6 (10.0%) | 27 (45.0%) | 0.001 | 5.824 (1.962-17.281) |
| | S/N | 32 (53.3%) | 16 (26.7%) | 0.327 | 0.647 (0.270-1.548) |
| | S/S | 22 (36.7%) | 17 (28.3%) | Reference | |
| | N | 44 (36.7%) | 70 (58.3%) | 0.001 | 2.418 (1.439-4.064) |
| | S | 76 (63.3%) | 50 (41.7%) | | Reference |
| UT21 Haplotypes | MN | 53 (44.2%) | 39 (32.5%) | 0.063 | 0.609 (0.360-1.029) |
| | MS | 19 (15.8%) | 24 (20%) | 0.400 | 1.329 (0.684-2.580) |
| UT89 Haplotypes | TN | 17 (14.2%) | 5 (4.2%) | 0.007 | 0.263 (0.094-0.739) |
| | TS | 31 (25.8%) | 52 (43.3%) | 0.004 | 2.195 (1.273-3.788) |

OR=Odds Ratio, 95% CI=95% confidence interval

**Table 3:** Comparison of genotypes, allele frequencies, and distribution of probable haplotypes of the UTS2 gene polymorphisms (Thr21Met and Ser89Asn) in cases with systemic sclerosis (SSc) and control.

The frequencies of NN, S/N and SS genotypes of the UT89 gene polymorphism were 45%, 26.7% and 28.3%, respectively in SSc patients. While their frequency in the control group were 10%, 53.3% and 36.7% respectively. A significant difference was observed in the NN genotype in SS patients compared to controls (p=0.001; OR=5.824; CI=1.962-17.281).

Regarding the Haplotypes of the UTS2, a significant difference was found in the haplotype TS between SSc cases (43.3%) and control subjects (25.8%) (p=0.004; OR=2.195; 95% CI=1.273-3.788), while haplotype TN was significantly higher in control subject (14.2%) than in SSc cases (4.2%) (p=0.007; OR=0.26; 95% CI=0.094-0.739) (Table 3).

Upon study the genotype frequency difference between the diffuse and limited subtypes, we found that; there were significant differences in the MM and NN genotypes in diffuse SSc patients (35.7%, 67.9% respectively) compared to limited SSc patients (6.2%, 25% respectively), (p=0.004; OR=8.333; 95% CI=1.638-42.395, p<0.001; OR=17.812; 95% CI=3.284-96.62 respectively). While the TM and SS genotypes showed higher frequency in the limited group (93.8%, 46.9% respectively) compared to diffuse (64.3%, 7.1% respectively) (p=0.004; OR=8.333; 95% CI=1.638-42.395, p<0.001; OR=6.382 95% CI=2.786-14.617) (Table 4).

| | | Limited scleroderma (32) N (%) | Diffuse scleroderma (28) N (%) | p value | OR (95% CI) |
|---|---|---|---|---|---|
| UT21 Genotype | M/M | 2(6.2) | 10(35.7) | 0.004 | 8.333 (1.638-42.395) |
| | T/M | 30(93.8) | 18(64.3) | | |
| | T/T | 0(0) | 0(0) | ....... | |
| | M | 34(53.1) | 38(67.9) | 0.1 | 1.863 (0.884-3.925) |
| | T | 30(46.9) | 18(32.1) | | |
| UT89 Genotype | N/N | 8(25.0) | 19(67.9) | <0.001 | 17.812 (3.284-96.623) |
| | S/N | 9(28.1) | 7(25.0) | 0.057 | 5.833 (0.988-34.436) |
| | S/S | 15(46.9) | 2(7.1) | Reference | |
| | N | 25(39.1) | 45(80.4) | <0.001 | 6.382 (2.786-14.617) |
| | S | 39(60.9) | 11(19.6) | | |

**Table 4:** Comparison of genotypes, allele frequencies, and distribution of probable haplotypes of the UTS2 gene polymorphisms (Thr21Met and Ser89Asn) in diffuse and limited scleroderma patients.

Regarding the association between the genotype and allele frequency with the clinical manifestations in SSc patients. It was found that the Patients carrying the Thr21Met MM genotype showed a significant association with pitting scars (p<0.001), digital ischaemia (p=0.001), Pulmonary hypertension (p=0.003) and GIT manifestations (p=0.007). While, Patients carrying the Thr21Met M allele showed a significant association with pitting scars (p=0.044). On the other hand, the patients carrying the Ser89Asn NN genotype showed a significant association with pitting scars (p<0.001), digital ischaemia (p=0.002), Pulmonary hypertension (p<0.001) and pulmonary manifestations (p<0.001). While, the patients carrying the Ser89Asn N allele showed a significant association with Raynauds phenomenon (p=0.002), pitting scars (p<0.001), digital ischaemia (p<0.001), GIT manifestations (p=0.022), pulmonary hypertension (p<0.001), pulmonary manifestations (p<0.001) and renal manifestations (p=0.013) (Tables 5 and 6).

| | | Pitting Scars | | | Raynauds Phenomenon | | | Digital Ischaemia | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | +ve Cases n=28 (%) | p value | OR (95%CI) | +ve Cases n=51(%) | p value | OR (95%CI) | +ve Cases n=30 (%) | p value | OR (95%CI) |
| UT21 Genotype | M/M | 11(39.3) | <0.001 | 20.059 (2.382-168.938) | 10(19.6) | 0.857 | 0.854 (0.153-4.752) | 11(36.7) | 0.001 | 16.789 (2.001-140.898) |
| | T/M | 17(60.7) | | | 41(80.4) | | | 19(63.3) | | |
| | T/T | 0(0) | | | 0(0) | | | 0(0) | | |
| | M | 39(69.6) | 0.044 | 2.155 (1.016-4.569) | 61(59.8) | 0.917 | 0.947 (0.339-2.644) | 41(68.3) | 0.062 | 2.019 (0.96-4.244) |
| | T | 17(30.4) | | | 41(41.2) | | | 19(31.7) | | |
| UT89 Genotype | N/N | 19(67.9) | <0.001 | 17.812 (3.284-96.623) | 25(49) | 0.089 | 5.208 (0.880-30.836) | 19(63.3) | 0.002 | 7.719 (1.918-31.058) |
| | S/N | 7(25) | 0.057 | 5.833 (0.988-34.436) | 14(27.5) | 0.398 | 2.917 (0.476-17.859) | 7(23.3) | 0.218 | 2.528 (0.568-11.259) |
| | S/S | 2 (7.1) | | | 12 (23.5) | | | 4(13.3) | Reference | |
| | N | 45(80.4) | <0.001 | 6.382 (2.786-14.617) | 64(62.7) | 0.002 | 3.368 (1.168-9.713) | 45(75) | <0.001 | 4.2 (1.93-9.141) |
| | S | 11(19.6) | | | 38(37.3) | | | 15(25) | | |

**Table 5:** Association of genotypes and allele frequencies of UTS2 gene Thr21Met and Ser89Asn polymorphism in systemic sclerosis cases with pitting scars, Raynauds phenomenon and digital ischaemia.

| | | Pulmonary hypertension | | | Pulmonary manifestations | | | Renal Manifestations | | | GIT Manifestations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cases n=32 (%) | p value | OR (95%CI) | Cases n=35 (%) | p value | OR (95%CI) | Cases n=14 (%) | p value | OR (95%CI) | Cases n=29 (%) | p value | OR (95%CI) |
| UT21 | M/M | 11(34.4) | 0.003 | 14.143 (1.68-118.42) | 9(25.7) | 0.190 | 2.538 (0.61-10.55) | 5(35.7) | 0.093 | 3.095 (0.79-12.02) | 10(34.5) | 0.007 | 7.632 (1.50-38.74) |
| | T/M | 21(65.6) | | | 26(74.3) | | | 9(64.3) | | | 19(65.5) | | |
| | T/T | 0(0) | | | 0(0) | | | 0(0) | | | 0(0) | | |
| | M | 43(67.2) | 0.086 | 1.906 (0.91-3.99) | 44(62.9) | 0.450 | 1.33 (0.63-2.78) | 19(67.9) | 0.331 | 1.553 (0.63-3.8) | 39(67.2) | 0.117 | 1.804 (0.85-3.87) |
| | T | 21(32.8) | | | 26(37.2) | | | 9(32.1) | | | 19(32.8) | | |
| UT89 | N/N | 23(71.9) | <0.001 | 18.688(3.99-87.49) | 21(60.9) | <0.001 | 11.375 (2.69-48.10) | 9(64.3) | 0.062 | 8.000 (0.91-70.27) | 18(62.1) | 0.096 | 2.857 (0.81-10.01) |
| | S/N | 5(15.6) | 0.708 | 1.477 (0.31-6.89) | 10(28.6) | 0.024 | 5.417 (1.19-24.52) | 4(28.6) | 0.175 | 5.333 (0.52-54.03) | 4(13.8) | 0.325 | 0.476 (0.10-2.10) |
| | S/S | 4(12.5) | | | 4(11.4) | | | 1(7.1) | | | 7(24.1) | | |
| | N | 51(79.7) | <0.001 | 7.64 (3.35-17.39) | 52(74.3) | <0.001 | 5.136 (2.33-11.29) | 22(78.6) | 0.013 | 3.316 (1.24-9.05) | 40(69) | 0.022 | 2.37 (1.12-5.00) |
| | S | 13(20.3) | | | 18(25.7) | | | 6(21.4) | | | 18(31) | | |

**Table 6:** Association of genotypes and allele frequencies of Thr21Met and Ser89Asn polymorphism in SSc cases with pulmonary hypertension, pulmonary, renal and GIT manifestations.

The comparison of autoantibodies between genotypes and alleles showed a significant association between Anti Sclero-70 Antibody and genotype UT21 MM (p<0.001) and UT21 allele M (p=0.043). While, no significant association was seen between UT21 genotypes/ alleles and the ANA (p=0.677 and 0.7 respectively) or ACA (p=0.327and 0.536 respectively). On the other hand, a significant association was found between Anti Sclero-70 Antibody and genotype UT89 NN (p=0.022) and UT89 allele N (p=0.005). While, No significant association was found between UT89 genotypes/alleles and the ANA (p=0.220 and 0.130, respectively) or ACA (p=0. 786 and 0.898, respectively) (Figures 1 and 2).
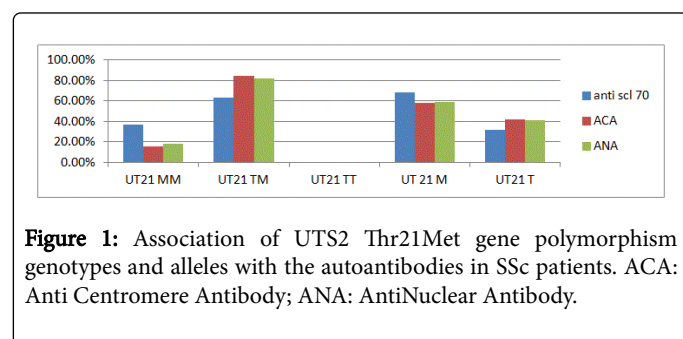


**Figure 1:** Association of UTS2 Thr21Met gene polymorphism genotypes and alleles with the autoantibodies in SSc patients. ACA: Anti Centromere Antibody; ANA: AntiNuclear Antibody.
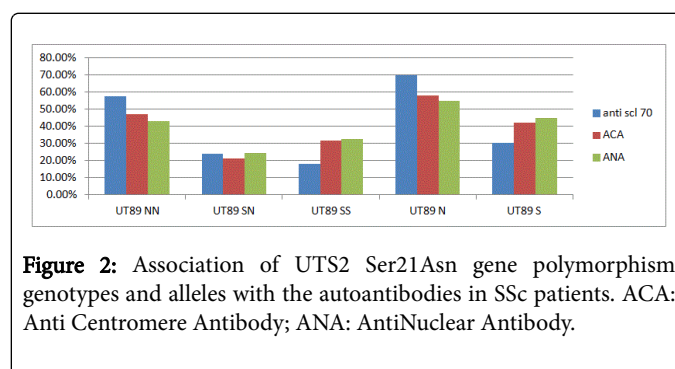


**Figure 2:** Association of UTS2 Ser21Asn gene polymorphism genotypes and alleles with the autoantibodies in SSc patients. ACA: Anti Centromere Antibody; ANA: AntiNuclear Antibody.

## Discussion

Urotensin II, being a potent vasoconstrictive agent, may play a role in the pathogenesis of SSc in many ways. Urotensin II has 50 times more vasoconstrictive effect on the arterial system and nearly ten times more effect on the venous system than Endothelin-1 (ET-1), a peptide proved to have a pivotal role from the early to late phases of SSc [4,14].

The aim of this study was to investigate the possible association of the polymorphism of UTS2 gene (Thr21Met and Ser89Asn) with the susceptibility to SSc and pattern of disease manifestations in Egyptian patients. To achieve this aim, PCR and RFLP techniques were applied. Association of genotypes and alleles to clinical manifestations and autoantibodies was investigated. We also compared genotypes and allele frequencies among limited SSc and diffuse SSc patients.

Patients with diffuse scleroderma showed significant higher rates of digital ischaemia (p=0.002), pitting scars (p<0.001), pulmonary hypertension (p=0.009), pulmonary manifestations in the form of dry cough and IPF (p=0.014), GIT manifestations in the form of dysphagia, reflux and dysmotility (p=0.021) and renal manifestations in the form of proteinuria (p=0.034). These results are similar to previous studies [17,18]. On the other hand, some studies proved that patients with limited SSc had more severe vascular disease, and suffered more from microcirculatory abnormalities leading to digital ischaemia (which is related to anti-centromere antibodies) [19].

Urotensin II (Thr21Met) genotyping in SSc patients revealed that the MM genotype is significantly higher in SSc patients compared to the control subjects. (p=0.013; OR=4.750; 95% CI=1.266-17.819). This is in accordance with Pehlivan et al. [14], who reported higher frequency of the Thr21Met polymorphism in SSc patients than in controls. In Pehvilan's study on Turkish population, the MM, the TM genotypes and the M allele were more common in SSc cases than in controls. Pehvilan also demonstrated marked association of SSc to MS and TS haplotypes. In our study on Egyptian population, the haplotype TS was significantly higher in SSc cases (43.3%) than in control subjects (25.8%) (p=0.004; OR=2.195; 95% CI=1.273-3.788), while haplotype TN was significantly higher in control subject (14.2%) than in SSc cases (4.2%) (p=0.007; OR=0.26; 95% CI=0.094-0.739).

Regarding the association between the clinical manifestations of SSc patients with different genotypes and alleles. We found that patients carrying the Thr21Met MM genotype showed a significant association with pitting scars (p<0.001), digital ischaemia (p=0.001), pulmonary hypertension (p=0.003) and GIT manifestations (p=0.007). Patients carrying the Thr21Met M allele showed a significant association with pitting scars (p<0.001) and digital ischaemia (p<0.001). This concurs with Pehlivan who reported that the MM genotype of Thr21Met polymorphism was associated with pitting scars, systemic involvement and lung involvement [14].

Urotensin II (Ser89Asn) genotyping in SSc patients revealed that the NN genotype is significantly higher in SSc patients compared to the control subjects. (p=0.001; OR=5.824; 95% CI=1.962-17.281). The frequency of the N allele of the UT89 gene polymorphism was significantly higher in the SSc patients (58.3%) than in the control subjects (36.7%). And the frequency of the S allele of the UT89 gene polymorphism was significantly higher in control subjects (63.3%) than in SSc patients (41.7%). (p=0.001; OR=2.418; 95% CI=1.439-4.064). These results were not compatible with the results of Pehlivan's study on Turkish population, which showed no association between Ser89Asn polymorphism of the UTS2 and SSc.

The frequency of the N allele of the UT89 gene polymorphism was significantly higher in diffuse SSc patients (80.4%) than in limited SSc patients (39.1%). While the frequency of the S allele of the UT89 gene polymorphism was significantly higher in limited SSc patients (60.9%) than in diffuse SSc patients (19.6%) (p≤0.001; OR=6.382; 95% CI=2.786-14.617).

Upon correlating the gene polymorphism to various clinical manifestations, we found that, the patients carrying the Ser89Asn NN genotype showed a significant association with pitting scars (p<0.001), digital ischaemia (p=0.002), Pulmonary hypertension (p<0.001) and pulmonary manifestations (p<0.001).

Patients carrying the Ser89Asn N allele showed a significant association with Raynauds phenomenon (p=0.002), pitting scars (p<0.001), digital ischaemia (p<0.001), GIT manifestations (p=0.022), pulmonary hypertension (p<0.001), pulmonary manifestations (P<0.001) and renal manifestations (p=0.013). Several studies indicated that Urotensin II levels were high in patients with diseases that show vasculopathies like diabetic retinopathy [20] and pre-eclampsia [21]. Urotensin II is also reported by many authors to cause fibrosis in many organs like liver [22,23], kidney [24], synovial fibrosis which is one of the main outcomes of osteoarthritis [25] and heart [26].

In conclusion, our findings suggest an association between UTS2 Thr21Met and Ser89Asn genetic polymorphisms and SSc susceptibility in the Egyptian population. Our results also associate these polymorphisms with the pattern of disease manifestations. The results strongly suggest that these 2 single-nucleotide polymorphisms may be important risk factors in the development of SSc, and an indicator of many disease manifestations such as pulmonary hypertension, severe skin and lung involvement in patients with SSc.

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