Study of urotensin-2 (T21M and S89N) gene polymorphisms in systemic sclerosis

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ABSTRACT

Background: Systemic sclerosis (SSc) is an immune-mediated fibrotic disease. Urotensin II (UTS2) is a new peptide with vasoactive and fibrogenic features, an association of UTS2 gene polymorphisms with SSc has been reported in the Turkish population.
Objectives: To study the possible association of UTS2 (T21M and S89N) gene polymorphisms with SSc in Egyptian patients.
Methods: 41 SSc patients and 70 healthy control subjects were genotyped for UTS2 (T21M and S89N) polymorphisms by TaqMan real-time PCR and PCR-RFLP, respectively.
Results: The frequency of the S/N genotype and N allele of UTS2 S89N were significantly higher in SSc patients as compared to the healthy controls ($p = 0.037$, OR 4.60; $p = 0.041$, OR 4.26, respectively). The Rodnan score was significantly higher in SSc patients carrying the combined (T/M + M/M) genotype of UTS2 T21M polymorphism as compared to those with the wild T/T genotype ($p = 0.018$).
Conclusion: UTS2 (T21M and S89N) gene polymorphisms may play an important role in the susceptibility and the clinical outcome of SSc in Egyptian patients.

1. Introduction

Systemic sclerosis (SSc) is a progressive and highly debilitating autoimmune disease characterized by inflammation, vasculopathy, and extensive fibrosis (Fuschiotti, 2016). It also represents one of the autoimmune systemic diseases with a worse prognosis and with high standardized mortality ratio 2.72 (Rubio-Rivas et al., 2014).

The etiology of SSc remains obscure, but it likely involves an interaction between environmental factors in a genetic predisposing background. Genetic linkage and genome-wide association studies have identified many polymorphisms associated with the predisposition of patients to have SSc (Ramos et al., 2015; Mahoney et al., 2015). These include genes of the major histocompatibility complex (MHC) class II (Jin et al., 2014) and also non-MHC genes (Pattanaik et al., 2015) as genes associated with the metabolism of extracellular matrix (ECM) molecule (Salazar and Mayes, 2015).

Pathogenesis of SSc is characterized by three hallmarks: vasculopathy of small vessels, altered innate and adaptive immunity, and extensive fibrosis of both the skin and visceral organs (Katsumoto et al., 2011).

There is a growing evidence that environmental factors have a significant role in both SSc onset and progression through modulation and alteration of the genetic determinants. A great correlation has been found between SSc onset and occupational exposure to crystalline silica and the following organic solvents: white spirit, aromatic solvents, chlorinated solvents, trichloroethylene, and ketones (Marie and Gehanno, 2015).

The SSc main feature is an excessive production and accumulation of collagen and other extracellular matrix proteins, leading to fibrosis and tissue dysfunction (Hunzelmann and Krieg, 2010), causing diverse patterns of clinical involvement that require an individualized approach to management (Denton, 2015).

Previously, therapeutics options in SSc were supportive of organ involvement. Nowadays, some drugs are used as a preventive of organ failure, i.e. angiotensin-converting enzyme (ACE) inhibitors and calcium channel blockers (Elhai et al., 2012).

Urotensin II (UTS2) is an 11-amino acid peptide derived as a result of proteolytic cleavage of two splice variants of preproprotein with 124 or 138 amino acids (Ross et al., 2010a), mediating its biological effects by interaction with the UTS2 receptor which belongs to the G-protein coupled receptor superfamily, originally termed GPR14 (G-protein-coupled receptor 14) (Liu et al., 1999; Mori et al., 1999; Nothacker et al., 1999). UTS2 has the most potent vasoconstrictor activity known

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yet (Yumrutas et al., 2015), with approximately 10-fold, 100-fold, and 300-fold greater potency than endothelin-1 (ET-1), serotonin (5-HT), and noradrenaline, respectively (Watanabe et al., 2009). The physiological mechanisms of which are similar in some ways to other potent mediators, as endothelin-1 (Ross et al., 2010b).

UTS2 could also exert a wide range of actions, such a proliferation of vascular smooth muscle cells, fibroblasts, and cancer cells. It can also enhance, inflammatory cells chemotaxis. On cardiac muscle, it has inotropic and hypertrophic effects. It has many other actions as inhibiting insulin release, modulating glomerular filtration, and affecting catecholamines release. Thus, UTS2 is proposed to contribute to various human diseases, including atherosclerosis, hypertension, cardiac hypertrophy, and diabetes (Ross et al., 2010b).

Previous studies reported that UTS2 could play a significant role in cardiovascular remodeling and fibrosis (Bousette and Giaid, 2006), hepatic fibrosis (Kemp et al., 2009), renal fibrosis (Tian et al., 2008), and that it increases collagen production (Tran et al., 2010). Pehlivan and his colleagues demonstrated that UTS2 was significantly elevated in patients with SSc (Pehlivan et al., 2011).

The gene for urotensin II (UTS2) is present in human chromosome 1p36.23. The US National Center for Biotechnology Information (NCBI) stated that over 60 single- nucleotide polymorphisms (SNPs) were noticed in the human UTS2 gene. Three of these SNPs show amino acid changes in the UTS2 gene sequence. High allelic frequencies in Japanese populations were documented for the T21M and S89N polymorphisms (Wenyi et al., 2003; Suzuki et al., 2004), and a Turkish study showed an association between T21M, but not S89N, in the UTS2 gene and SSc (Pehlivan et al., 2012).

T21M is a substitution variant in which nucleotide transition from C to T in codon 61 of the UTS2 gene, results in an amino acid transition from Threonine to Methionine at amino acid position 21. While in S89N polymorphism, there is a nucleotide transition from G to A in codon 266 which resulted in an amino acid transition from Serine to Asparagine at amino acid position 89 (Wenyi et al., 2003).

The pathogenesis of SSc is characterized mainly by microangiopathy, defective angiogenesis, immune dysfunction, and progressive fibrosis of the skin and internal organs (Varga and Abraham, 2007; Manetti et al., 2010). Exploration of new genetic factors could help to reveal the pathogenesis of vascular, autoimmune, and fibrotic manifestations for the development of new therapeutic strategies.

The goal of this study was to detect the possible association of UTS2 (T21M and S89N) gene polymorphisms with SSc in Egyptian patients.

2. Materials and methods

This study included 41 SSc patients and 70 age & sex matched healthy control volunteers. SSc patients were recruited from the outpatient clinic of the Rheumatology department at Kasr Al Aini hospitals and subjected to full clinical examination and immunological investigations (antinuclear, anticentromere, antiscleroderma-70 autoantibodies). All SSc patients fulfilled the 2013 American College of Rheumatology (ACR) classification criteria for systemic sclerosis (Van den Hoogen et al., 2013). They were evaluated for disease pattern (limited or diffuse); clinical involvement of different organs: musculoskeletal, cardiovascular, pulmonary, gastrointestinal and renal involvement. Skin involvement was assessed using Rodnan score (Clements et al., 1995). Table 1 shows the descriptive data of SSc patients. The healthy control group included 60 (85.7%) females and 10 males (14.3%) with mean age 38.2 ± 8.4 years.

3. Blood samples

Five milliliter venous blood was withdrawn from all subjects and divided into 2 tubes: Two milliliter in EDTA tube stored in −20 °C till the time of DNA isolation and 3 ml in a plain sterile tube for autoantibodies detection.

Table 1
Descriptive data of scleroderma patients (n = 41).

	Scleroderma patients (n = 41)
Age (years)	39.5 ± 11.8
Female sex, n (%)	35 (85.4)
Disease duration (years)	6.0 (3.5–12.0)
Rodnan score	12.0 (11.0–14.5)
Subtypes, n (%)	
Diffuse	9 (22.0)
Limited	32 (78.0)
Autoantibodies, n (%)	
ANA	32 (78.0)
ACA	19 (46.3)
Scl70	7 (17.1)
Clinical manifestation, n (%)	
Raynaud's phenomenon	39 (95.1)
Digital ulcers	15 (36.6)
Pitting scars	24 (58.5)
Digital gangrene	9 (22.0)
Acroosteolysis	10 (24.4)
Arthritis	14 (34.1)
Myositis	7 (17.1)
Dysphagia	30 (73.2)
Dyspnea	30 (73.2)
IPF	24 (58.5)
Pulmonary hypertension	5 (12.2)
Treatment, n (%)	
Steroids and immunosuppressives	28 (68.3)
Steroids	6 (14.6)
Vasodilators and ppi	4 (9.8)
Vasodilators	3 (7.3)

ANA, antinuclear antibodies; ACA, anticentromere antibodies; Scl70, anti-topoisomerase I antibodies; IPF, interstitial *pulmonary fibrosis; PPi, proton pump inhibitors.* Variables with normal distribution are presented as mean ± standard deviation. Skewed variables are presented as median (interquartile range).

4. Immunological investigations

SSc patients' sera were tested for the presence of antinuclear antibodies (ANA) by indirect immunofluorescence (IF). Anticentromere antibodies (ACA) were detected by IF using HEp-2 cells. Antitopoisomerase I antibodies (Anti-Scl70) were determined using ELISA technique (Alegria automated system, ORGENTEC Diagnostika, Germany).

5. DNA isolation and UTS2 genotyping

DNA was extracted from patients and controls peripheral blood cells using G-spin Total DNA Extraction Mini Kit (INTRON Biotechnology, Korea). Two single nucleotide polymorphisms (SNPs) of UTS2 (T21M, rs228648) and (S89N, rs2890565) were analyzed.

UTS2 (T21M, rs228648) polymorphism was genotyped using TaqMan real-time PCR SNP assay. The pre-designed SNP was purchased from Applies Biosystems. The thermal cycling conditions were: 95 °C for 10 min, 50 cycles of 92 °C for 10 s and 60 °C for 1 min. Data analysis for allelic discrimination was performed with the Applied Biosystems step one Real-Time PCR System software (Applied Biosystems, Foster City, CA, USA).

UTS2 (S89N, rs2890565) polymorphism was genotyped using PCR-Restriction Fragment Length Polymorphism (RFLP) analysis. A 263 bp PCR amplification product was generated using the following primers 5′- GTGCCTGTCTGTCTGCATTCA-3′ (Forward) and 5′-GAGTCCTG TAAAACCAGTACAG-3′ (Reverse). After an initial denaturation at 95 °C for 5 min, 32 PCR cycles were performed (95 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s), followed by an elongation of 72 °C for 10 min. The UTS2 S89 N polymorphism was analyzed by digestion of the PCR product with RsaI (New England Biolabs Inc., MA, UK) which resulted in three fragments of 161, 84, and 18 bp in the presence of the 89S allele, while the 89N allele showed two fragments of 245 and 18 bp (Okumus et al., 2012). All PCR reactions were carried out in a Biometra