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Research Paper

FOXP3, *ICOS* and *ICOSL* gene polymorphisms in systemic sclerosis: *FOXP3* rs2294020 is associated with disease progression in a female Italian population

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ABSTRACT

Systemic sclerosis (SSc), an autoimmune disorder, is characterized by vasculopathy, inflammation, progressive perivascular and interstitial fibrosis. Its pathogenesis is largely unknown, however strong evidences suggest that genetic predisposition may contribute to SSc development.

Several gene polymorphisms involved in regulatory T cell function have been identified in many autoimmune diseases, including SSc. Moreover, dysregulation of co-stimulatory and/or co-inhibitory signals, including ICOS signalling, can lead to autoimmunity. The aim of the present study was to investigate the association of the FOXP3 rs2294020, ICOS rs6726035 and ICOSL rs378299 SNPs with both the susceptibility and the progression to SSc in an Italian case-series of patients.

SNP genotyping results were successfully obtained from a total of 350 subjects including 166 individuals with SSc and 184 healthy controls. Although analysis tests did not show any significant associations between the SNPs under study and susceptibility to SSc, the occurrence of *FOXP3* rs2294020 in female patients was associated with decreased time to progression from early to definite SSc (allelic model: HR = 1.43; CI = 1.03-1.99; p = 0.03; dominant model: HR = 1.54; CI = 1.04-2.28; p = 0.03). The inclusion of presence of ACA autoantibodies in the model did not significantly change the estimates. No conclusions can be drawn for the susceptibility to the disease or the time to progression in men due to the low statistical power. This study provides evidence of the association of rs2294020 with SSc evolution in female patients, modulating the time of progression from the diagnosis of early SSc to the diagnosis of definite SSc, while no effect on SSc susceptibility per se was found. rs2294020 may be considered a disease-modifying gene-variant rather than a disease-susceptibility SNP in SSc.

1. Introduction

Systemic sclerosis (SSc), an autoimmune disorder associated with substantial morbidity and mortality rates (Nikpour et al., 2010), is characterized by vasculopathy, inflammation, progressive perivascular and interstitial fibrosis (Desbois and Cacoub, 2016). SSc pathogenesis is largely unknown, but there is growing evidence suggesting a close connection between environmental and genetic factors (Barsotti et al., 2016).

T regulatory (Treg) population, a specialized T cell subpopulation, are known to play an important role in controlling and limiting harmful immune responses, such as autoimmunity (Li and Rudensky, 2016). Among the different cell subpopulations of natural FOXP3$^+$Treg cells, there exist two subsets of this subpopulation according to the differential expression of the costimulatory receptor ICOS: ICOS$^+$FOXP3$^+$Tregs, which show suppression activity through IL-10 and TGFβ production, and ICOS$^-$FOXP3$^+$Tregs, which predominantly use TGFβ (Ito et al., 2008). In particular, ICOS$^+$FOXP3$^+$ Treg population plays a pivotal role in controlling skin inflammation, with a significant involvement of ICOS for the antigen-specific suppression

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Table 1
Baseline demographic and clinical characteristics of SSc patients and healthy control subjects (abbreviations: SEM, standard error of mean; Scl70, anti-topoisomerase; ACA, anti-centromere antibody; * values in parentheses refer to SSc patients of the definite form).

Variable	Value	
	Patients	Healthy controls
Age (years) of diagnosis of early SSc, mean (± SEM)	48.09 (± 1.09)	40.69 (± 1.52)
Males	5.16%	27.72%
Definite disease form	69.06%	–
Disease evolution time (months) from *early* to *definite* form, median (95% confidence interval)	47 (32–58)	–
ANA non specific antibody, % (*)	9.03 (9.35)	–
ANA nucleolar antibody, % (*)	10.32 (55.14)	–
ACA antibody, % (*)	61.93 (21.49)	–
Scl70 antibody, % (*)	18.06 (12.15)	–

(Vocanson et al., 2010).

Specifically expressed in $CD4^+CD25^+$Treg cells, forkhead box P3 (FOXP3) is required for the development of this cell subset (Fontenot et al., 2003). FOXP3 is a member of the forkhead transcription factor family encoded by the *FOXP3* gene located on the X chromosome (Plitas and Rudensky, 2016). FOXP3 is responsible for the transcriptional regulation of genes involved in Treg phenotypic and functional signature (Grant et al., 2015; Haiqi et al., 2011). Alterations in Treg numbers and/or function may contribute to autoimmune diseases, including SSc (Kataoka et al., 2015; MacDonald et al., 2015). Decreased numbers of Tregs, as well as a downregulation of FOXP3, were associated with SSc (Antiga et al., 2010; Wang et al., 2014).

Inducible T cell co-stimulatory (ICOS) is a type-I transmembrane molecule that shows a close structural resemblance to CD28 and CTLA-4 (Hutloff et al., 1999). Together with CD28 and CTLA-4, ICOS is expressed on activated $CD4^+$ and $CD8^+$ T cells, underlining its involvement in the adaptive T cell response to a foreign antigen (Watanabe et al., 2005). Its expression is rapidly induced after TCR cross-linking and/or CD28 co-stimulation (Wikenheiser and Stumhofer, 2016). ICOS binds to its ligand ICOSL, mainly expressed on B cells, dendritic cells and macrophages (Yoshinaga et al., 1999). The interaction of ICOS with ICOSL is required for the major activities of T cells, including cytokine production and their differentiation into T follicular helper cells and Th17 (Leconte et al., 2016). Moreover, ICOS-ICOSL interaction may influence the expansion and activation of Tregs (Burmeister et al., 2008; Zheng et al., 2013). As a result of ICOS/ICOSL interaction, naïve $CD4^+$ T cells can differentiate into IL-10–producing Treg (Ito et al., 2007), which are known to play a pivotal role in autoimmune diseases, including SSc (Liu et al., 2016). Moreover, medullary thymus epithelial cells, through ICOSL signalling, can induce the production of IL-2 by CD25- T cells, thus leading to the expansion of tTregs (Nazzal et al., 2014).

It is known that dysregulation of co-stimulatory and/or co-inhibitory signals, including ICOS signalling, can cause a breakdown of self-tolerance, thus leading to autoimmunity (Zhang and Vignali, 2016). Recently, it has been reported that ICOS expression levels are increased in both peripheral blood memory T cells and Tregs in patients affected by SSc, thus suggesting that ICOS signalling may contribute to the pathogenesis of SSc (Hasegawa et al., 2013).

It has been reported that ICOSL may play a role, independently of the ICOS interaction, in a mouse model of bleomycin-induced lung and skin fibrosis (Tanaka et al., 2010).

Several polymorphisms located in genes mainly involved in MHC-antigen presentation, TCR, BCR signalling, and Treg function, have been identified in many autoimmune diseases, including SSc (Gruschwitz et al., 1991; Kochi, 2016). In particular, several genetic variants that influence Treg activity have been identified (Oda et al., 2013; Tao et al., 2017).

The aim of the present study was to investigate the association of the *FOXP3* rs2294020, *ICOS* rs6726035 and *ICOSL* rs378299 SNPs with both the susceptibility and the progression to SSc in an Italian case-series of SSc patients.

2. Materials and methods

2.1. Patients and control subjects

The study, designed as a case-control study, was composed of 350 unrelated Italian subjects (280 women and 70 men), with a total of 166 (147 women and 19 men) SSc cases and 184 healthy controls (133 women and 51 men). All patients and healthy subjects were recruited in a single tertiary referral center (Scleroderma Unit, Fondazione IRCCS Ca' Granda Policlinico di Milano). Patients fulfilled the 2013 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria (van den Hoogen et al., 2013) or the criteria for the diagnosis of *Early SSc* (EaSSc) proposed by LeRoy and Medsger (2001). At the time of the first visit, all the study subjectsd were analyzed for anticentromere (ACA), anti-topo I (Scl70) and antinuclear (ANA) antibodies, using indirect immunofluorescence staining and ELISA technique. All the study participants performed nailfold videocapillaroscopy/NVC) according to recommended guidelines (Cutolo et al., 2000). The time from evolution from EaSSc to definite SSc up to 10 years (120 months) from referral, was reconstructed reviewing the medical chart as described elsewhere (Vigone et al., 2015). All subjects gave informed consent for the study, which was approved by the local ethics committee. The clinical characteristics of the enrolled subjects are summarized in Table 1.

2.2. SNP selection

On the basis of Ensembl linkage disequilibrium data (www.ensembl.org), the variant rs2294029 (747A > G) was identified as a tag polymorphism for *FOXP3* gene with a minor allele frequency (MAF) > 0.01. The SNP is located on chromosome X (49246763, forward strand). This variant overlaps 9 transcripts: 8 downstream gene variants for *FOXP3* and 1 synonymous variant for *CCDC22* (coiled-coil domain containing 22). rs2294020 variant is positioned in the putative 3′-UTR region of *FOXP3* gene and in close proximity to the *CCDC22* gene in the complementary strand (exon 7 of the coding *CCDC22*, http://www.ensembl.org).

ICOS rs6726035 and *ICOSL* rs378299 were selected based on selection criteria from literature data on association analysis in other autoimmune diseases (Kim et al., 2010; Conteduca et al., 2014). The rs6726035 (12:g.204251279T > C) is a 3-near gene variant of *ICOS*, positioned in chromosome 2. The rs378299 (9:g.44241460C > T) is an upstream gene variant of *ICOSL*, located in chromosome 21.

2.3. DNA extraction and genotyping

Genomic DNA was extracted from whole peripheral blood with a commercial DNA isolation kit (Nuclear Laser Medicine, Italy), with a salting out method. Genotype analysis was performed by high-