

## No evidence for a role of the proximal IL-6 G/C -174 single nucleotide polymorphism in Italian patients with systemic sclerosis

Dear Editor,

In a recent issue of the JCOMM, Sfrent-Cornateanu *et al.* [1] described an association between the G/A single nucleotide polymorphism (SNP) in the position -597 of the IL-6 gene, with disease activity and disability, but not with the disease per se, in 20 systemic sclerosis (SSc) patients. The idea of a disease-modifying, -rather than a disease-susceptibility, point mutation is intriguing and in line with others findings in the context of SSc [2–5]. Nonetheless, the inability to depict such an association could be attributable to the low number of subjects and indeed the possibility of a type II error in such a small group of patients is more than remote. Based on previous findings that indicate that the G/C substitution at position -174 in complete

linkage disequilibrium with the G/A-597 SNP-, is associated with an increased IL-6 production *in vitro* and *in vivo* [6], the authors anticipated a decreased risk for SSc in CC -174 (AA -597) individuals compared to GG -174 (GG -597) subjects and claimed further studies in larger patient groups to verify this hypothesis and their findings as well.

The present retrospective case-control study was conducted on 196 consecutive patients with a diagnosis of SSc (ACR criteria [7]), referring to our outpatient clinic. One-hundred-ninety-six healthy sex-, age- and ethnically-matched subjects were included as controls. The number of patients and the case-to-control ratio were chosen so as to obtain a power of

**Table 1** Interleukin-6 G/C -174 single nucleotide polymorphism in 196 Italian systemic sclerosis patients and 196 healthy ethnically-matched controls

Variable	Genotype			Allele	
	GG	CG	CC	G	C
Controls, n (%)	89 (45.4%)	87 (44.4%)	20 (10.2%)	265 (67.6%)	127 (32.4%)
SSc, n (%)	84 (42.9%)	94 (48%)	18 (9.2%)	262 (66.8%)	130 (33.2%)
lcSSc	63 (44.4%)	68 (47.9%)	11 (7.7%)	194 (68.3%)	90 (31.7%)
dcSSc	21 (38.9%)	26 (48.1%)	7 (13%)	68 (63%)	40 (37%)
HAQ-DI, score	0.86±0.77	0.67±0.6	1.2±0.58	0.79±0.71	0.8±0.63
ESsSG, score	1.52±1.25	1.71±1.21	1.33±1.7	1.58±1.27	1.61±1.18
VAS, mm	38.3±30.1	42.5±28.7	42±22.4	39.8±29.3	42.4±26.8
FVC, % pred	82.7±23.7	81.2±20.8	77.8±24.9	82.2±22.5	80.2±21.8
DLco, % pred	56.2±19.6	57.2±19.6	57.2±18.6	56.7±19.5	57.4±19.2
PAP, mmHg	32.2±18	29±14.2	38.9±17.7	30.1±16.7	31.6±15.6
mRSS	5.3±5	5.7±5.6	6.7±4.7	5.5±5.2	6±5.3

Variables expressed as mean ± standard deviation, except where otherwise indicated. Statistical analysis performed by the chi-square test, Student's t-test (between alleles) or ANOVA (among genotypes); *P-value* not significant for all the comparisons. SSc, systemic sclerosis, -lcSSc, limited- and -dcSSc, diffuse- [10]. HAQ-DI, Health Assessment Questionnaire Disability Index [11]; ESsSG, European Scleroderma Study Group activity score [12]; VAS, visual analog scale (1–100 mm); FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide; PAP, pulmonary systolic pressure; mRSS, modified Rodnan Skin Score [13].

0.75 with an alpha level equal to 0.05 with a putative difference of 9% in the prevalence of the G allele at position -174; power calculation was carried out with the PS Program [8] and by using the frequencies of the G/C -174 SNP previously published in the Italian population as reference [9]. Clinical procedures for the study were those described by Sfrent-Cornateanu [1]: patients were categorized as having the limited cutaneous (lcSSc) or the diffuse cutaneous (dcSSc) subset of the disease according to LeRoy *et al.* [10] and were asked to complete the Disability Index of the Health Assessment Questionnaire (HAQ-DI) [11]; disease activity was assessed according to the European Scleroderma Study Group (ESsSG) [12] and by an experienced physician (AS) on a 100 mm visual analogue scale (VAS). The G/C -174 SNP was determined with sequence-specific primers by a commercial kit (CTS•PCR-SSP TRAY, from the Institute of Immunology, Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany) as previously described [4, 5, 9]. All the participating subjects gave written consent for the research. Statistics were carried out with the SPSS package, ver 15.0 (SPSS Chicago, IL).

The majority of patients were females (n = 178, 90.8%), with the lcSSc subset of the disease (n = 142, 72.4%), with a mean age and disease duration of 59.6±12.4 and 11.2±6.8 years, respectively. The distribution of the G/C-174 genotypes in patients and controls was consistent with Hardy-Weinberg's equilibrium; the genotypes or the alleles of the G/C -174 SNP were equally distributed between cases and controls (Table). HAQDI, VAS or ESsSG activity scores, as well as the most recent forced vital capacity % of predicted values, diffusing capacity for carbon monoxide % of predicted values, pulmonary systolic pressure on echocardiogram and total skin scores [13] were equally distributed among the G/C -174 genotypes or alleles (Table).

The G/C -174 (G/A -597) SNP seems thus to play no role either in SSc-susceptibility or in SSc activity or disability, as suggested by Sfrent-Cornateanu *et al.* [1]. The latter results are not unexpected, as functional (*e.g.* HAQ-DI) and activity scores (*e.g.* ESsSG and VAS) are "dynamic" measures that reflect the patient's short term condition (*e.g.* within 1–4 weeks) and may

change over time and in response to therapy [14, 15], being hence inadequate for comparison with a "static" genetic variable. Of course, at the moment we cannot definitively rule out the possibility that the G/C -174 (G/A -597) SNP might be associated with other aspects of the disease when a more thorough analysis is carried out in a prospective fashion on selected outcomes variables or in selected subgroup of patients, even if this possibility seems quite remote.

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## Authors' Reply

Dear Editor,

We appreciate Dr Beretta and co-workers' interest in our report. Dr Beretta's study confirmed a part of our conclusions: in our short communication no statistical significant differences were found between SSc patients and healthy controls with respect to the -597 (-174) allele distribution [1]. The data communicated by Dr Beretta are based on a valid power calculation and therefore rule out, with a probability of 75%, the possibility of a statistically significant difference of 9% or higher of the prevalence of the G allele in the position -174 of the IL-6 promoter between healthy controls and SSc patients from Italy. Our short communication presented the data of a pilot-study, whose aim was to investigate any possible association between the 597 single nucleotide polymorphism (SNP) of the IL-6 promoter and either the susceptibility to SSc

or any clinical characteristics of this disease. Following these preliminary data, our group is about to complete a larger, multicentric study on the association of SSc and IL-6 genetic polymorphisms and we shall soon be able to publish new data, sustained by a satisfactory power calculation.

Using the same method as in our report [1], the larger study by Beretta *et al.* failed to find any association of the G allele in the position -174 of the IL-6 promoter with higher disease activity and disease-induced disability. Due to the large number of subjects in the Italian cohort, this lack of association is very improbable to be due to a type II error.

We completely agree that HAQ-DI and EScSG disease activity score are dynamic measures that vary in the course of the disease. However, we consider

that these scores, and especially the HAQ-DI, reflect the patients' condition on a larger period of time.

In the study of Clements *et al.* on the value of the HAQ-DI as a predictor and correlate of outcome in their D-penicillamine trial in SSc patients, the mean change in HAQ-DI over 2 years in 68 patients who completed the study (all having received active treatment in two different doses) was 0.17, approximately 17% of the mean baseline value [2]. In the large cohort of Steen and Medsger, including 1250 SSc patients, there was little change in the mean HAQ-DI over time in the group as a whole, and only selected subsets showed significant and meaningful changes in HAQ-DI within 3 months [3]. It has been shown by Clements and coworkers that impaired fist closure and reduced handspread are among the main correlates of disability in SSc [4], which suggest that at least part of the HAQ-DI accounts for damage produced by the disease. In the absence of strong longitudinal data, allowing an analysis of genetic influence on disease course, it is thus not senseless to study the influence of the -597 IL-6 pr polymorphism on the HAQ-DI.

As for the EScSG activity score, we couldn't find any studies on its changes over time. It is already known that SSc patients have greater disease activity during the first years since disease onset [4] although this hasn't been studied by the means of the EScSG disease activity score.

Even if the clinical parameters chosen for the polymorphism gene association (HAQ-DI, EScSG) could be discussed, both studies took into account these parameters. The difference in the results of the two studies might also appear due to demographic differences between the two groups of SSc patients. Our study described a group of younger patients ( $44.4 \pm 10.7$  years *versus*  $59.6 \pm 12.4$  years), with a shorter disease duration ( $6.8 \pm 6.2$  years *versus*  $11.2 \pm 6.8$  years) than the group of patients analyzed in Dr Beretta's study.

We agree with Dr Beretta's conclusions that at the moment we can not definitively rule out the possibility that the G/C -597 (-174) SNP might be associated with different aspects of the disease. The influence of the -597 IL-6 pr polymorphism on disease activity and disease-induced disability may be confirmed by longitudinal studies, showing a different disease

course on the long-term between subjects with different genotypes, in order to prove the influence of the above-mentioned polymorphism on disease prognosis. Such a study has to be carried out prospectively, as the evaluation of disability and disease activity cannot be performed on retrospective patient data, to ensure sufficient follow-up (at least 1–2 years) and of course to have sufficient statistical power.

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