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BRIEF REPORT

Candidate Gene Study in Systemic Sclerosis Identifies a Rare and Functional Variant of the *TNFAIP3* Locus as a Risk Factor for Polyautoimmunity

Eugénie Koumakis,[1] Matthieu Giraud,[2] Philippe Dieudé,[3] Vanessa Cohignac,[2] Giovanna Cuomo,[4] Paolo Airò,[5] Eric Hachulla,[6] Marco Matucci-Cerinic,[7] Elizabeth Diot,[8] Paola Caramaschi,[9] Luc Mouthon,[10] Valeria Riccieri,[11] Jean-Luc Cracowski,[12] Kiet Phong Tiev,[13] Camille Francès,[14] Zahir Amoura,[15] Jean Sibilia,[16] Anne Cosnes,[17] Patrick Carpentier,[18] Gabriele Valentini,[4] Mirko Manetti,[7] Serena Guiducci,[7] Olivier Meyer,[19] André Kahan,[10] Catherine Boileau,[20] Gilles Chiocchia,[2] and Yannick Allanore[1]

Objective. **Systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) share some pathophysiologic bases as evidenced by individual and familial polyautoimmunity and common susceptibility genetic factors. With regard to the latter, there has been a recent shift from the "common variant" to the "rare variant" paradigm, since rare variants of *TNFAIP3* and *TREX1* with large effect sizes have recently been discovered in SLE. The present study was undertaken to investigate whether rare variants of *TNFAIP3* and *TREX1* are also associated with SSc.**

Methods. ***TREX1*** **single-nucleotide polymorphisms (SNPs) rs3135946, rs7626978, rs3135943, and rs11797 and *TNFAIP3* SNPs rs9494883, rs72063345, rs5029939, rs2230926, rs117480515, and rs7749323 were genotyped in a discovery set (985 SSc patients and 1,011 controls), and replication analysis of the most relevant results was performed in a second set (622 SSc patients and 493 controls).**

Results. **No association between *TREX1* variants and SSc was observed. For *TNFAIP3*, we first demonstrated that a low-frequency variant, rs117480515, tagged the recently identified TT>A SLE dinucleotide. In the discovery sample, we observed that all tested *TNFAIP3* variants were in linkage disequilibrium and were associated with SSc and various SSc subsets, including the polyautoimmune phenotype. We subsequently genotyped rs117480515 in the replication sample and found it to be associated solely with the SSc polyautoimmune subset (odds ratio 3.51 [95% confidence interval 2.28–5.41], $P = 8.58 \times 10^{-9}$) in the combined populations. Genotype–messenger RNA (mRNA) expression correlation analysis revealed that the *TNFAIP3* rs117480515 risk allele was associated with decreased mRNA expression.**

Conclusion. **The present findings establish the *TNFAIP3* locus as a susceptibility factor for the subset of SSc with a polyautoimmune phenotype. Our results support the implication of rare/low-frequency functional variants and the critical role of A20 in autoimmunity.**

Evidence of a shared pathogenic basis across connective tissue diseases (CTDs) is provided by familial

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[1]Eugénie Koumakis, MD, Yannick Allanore, MD, PhD: Paris Descartes University, INSERM U1016, Institut Cochin, and Cochin Hospital, AP-HP, Paris, France; [2]Matthieu Giraud, PhD, Vanessa Cohignac, Gilles Chiocchia, PhD: Paris Descartes University, INSERM U1016, and Institut Cochin, Paris, France; [3]Philippe Dieudé, MD, PhD: Paris Diderot University, INSERM U699, and Hôpital Bichat Claude Bernard, AP-HP, Paris, France; [4]Giovanna Cuomo, MD, Gabriele Valentini, MD: Second University of Naples, Naples, Italy; [5]Paolo Airò, MD: Spedali Civili, Brescia, Italy; [6]Eric Hachulla, MD, PhD: Université Lille II, Lille, France; [7]Marco Matucci-Cerinic, MD, PhD, Mirko Manetti, PhD, Serena Guiducci, MD, PhD: University of Florence, Florence, Italy; [8]Elizabeth Diot, MD, PhD: INSERM U618, IFR 135, Centre Hospitalier Universitaire Bretonneau, Tours, France; [9]Paola Caramaschi, MD: University of Verona, Verona, Italy; [10]Luc Mouthon, MD, PhD, André Kahan, MD, PhD: Paris Descartes University and Cochin Hospital, AP-HP, Paris, France; [11]Valeria Riccieri, MD: Sapienza University of Rome, Rome, Italy; [12]Jean-Luc Cracowski, MD, PhD: INSERM CIC3, Centre Hospitalier Universitaire Grenoble, Grenoble, France; [13]Kiet Phong Tiev, MD, PhD: Université Pierre et Marie Curie and Hôpital St. Antoine, AP-HP, Paris, France; [14]Camille Francès, MD: Université Paris 6 and Hôpital Tenon, Paris, France; [15]Zahir Amoura, MD: Université Paris 6 and Hôpital Pitié Salpétrière, Paris, France; [16]Jean Sibilia, MD, PhD: Université Louis Pasteur and Hôpital Hautepierre, Strasbourg, France; [17]Anne Cosnes, MD: CHU Créteil, AP-HP, Paris, France; and Hôpital Henri Mondor, Créteil, France; [18]Patrick Carpentier, MD: Centre Hospitalier Universitaire Grenoble, Grenoble, France, [19]Olivier Meyer, MD: Paris Diderot University and Hôpital Bichat Claude Bernard, AP-HP, Paris, France; [20]Catherine Boileau, PhD: Université Versailles Saint Quentin Yvelines and Hôpital Ambroise Paré, AP-HP, Boulogne, France.

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Address correspondence to Yannick Allanore, MD, PhD, Hôpital Cochin, Service de Rhumatologie A, 27 Rue du Faubourg Saint Jacques, 75014 Paris, France. E-mail: yannick.allanore@cch.aphp.fr.

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and individual aggregation of autoimmune diseases and by the well-known pleiotropism of autoimmune susceptibility genes (1,2). In recent years, a number of novel and unbiased genetic associations have been revealed, notably through genome-wide association studies (GWAS) (3). The underlying rationale for GWAS is the "common disease, common variant" hypothesis, positing that common diseases, such as autoimmune diseases, are attributable in large part to allelic variants (single-nucleotide polymorphisms [SNPs]) present in >5% of the population. However, the attributable risk conferred by the susceptibility alleles identified to date explains only 15–20% of heritability (4). There is now evidence that a number of low-frequency (i.e., 1–5%) and rare (<1%) variants that have larger effect sizes and more functional consequences with regard to phenotypic effects than do common variations are involved in the genetic susceptibility to complex traits, thus resulting in a shift from the "common variant" hypothesis to the "rare variant" paradigm (5). In systemic lupus erythematosus (SLE), rare alleles with a major effect on disease susceptibility have recently been discovered (6). Among these, mutations in *TREX1* and low-frequency variants in *TNFAIP3* have been highlighted (7–10).

TREX1 (3p21.31), the gene for 3′ repair exonuclease 1 (Trex1), encodes the major 3′→5′ DNA exonuclease involved in a proofreading function during genome replication. There is evidence that Trex1 might be a negative regulator of the interferon-stimulatory DNA response and that it could minimize immune activation by self DNA and prevent cell-intrinsic initiation of autoimmune processes. *TNFAIP3* (6q23.3), the tumor necrosis factor α–inducible protein 3 gene, encodes A20, a zinc-finger protein required for inhibition of the NF-κB signaling axis. Recently, several polymorphisms in the *TNFAIP3* locus have been found to be associated with increased susceptibility to a number of autoimmune diseases, i.e., SLE, rheumatoid arthritis, Crohn's disease, psoriasis, multiple sclerosis, type 1 diabetes, and systemic sclerosis (SSc) (9,11,12).

SSc is a rare multisystem autoimmune disease classified as a CTD. Numerous genetic factors underlying susceptibility to SSc have been identified, the vast majority belonging to autoimmune pathways. Among these genetic factors, the *TNFAIP3* SNP rs5029939, located in the second intron, was recently identified by our group as being strongly associated with SSc, with the strongest effect size reported to date in SSc outside the HLA locus (odds ratio [OR] >2) (12). However, the *TNFAIP3* causal variant still remains to be identified, and functional data are needed to clarify its functional consequences. A recent genetic study in SLE identified a functional TT>A dinucleotide variant, located 41.5 kb downstream of the *TNFAIP3* promoter, that could be the causal variant (10).

Given the evidence of a shared genetic background between SSc and SLE and the emerging rare variants paradigm, we investigated the association of low-frequency and rare variants of the *TREX1* and *TNFAIP3* gene regions, identified as susceptibility factors for SLE, with SSc and disease subsets. In addition, we performed genotype–messenger RNA (mRNA) expression correlation analyses to assess their putative functional implications.

PATIENTS AND METHODS

Study population. We performed a case–control association study, using a French Caucasian population consisting of 841 SSc patients and 997 healthy unrelated ethnically matched controls as previously described (13) for the genotyping of *TREX1* variants, and an extended French cohort consisting of 1,043 SSc patients and 1,095 matched controls for the genotyping of *TNFAIP3* variants. The replication set was an Italian cohort comprising 668 SSc patients and 501 controls. We included only individuals of European ancestry (defined as all 4 grandparents being of European Caucasian ancestry). Characteristics of the SSc patients in both cohorts are shown in Table 1. In addition, both French and Italian SSc patients were investigated by local physicians for the co-occurrence of autoimmune diseases known to be associated with CTDs, which were identified in 268 of them (15.7%). These autoimmune diseases were SLE, rheumatoid arthritis, Sjögren's syndrome, autoimmune thyroid disease, and primary biliary cirrhosis, and were classified in accordance with standard international criteria. All patients were screened for antinuclear antibodies

Table 1. Characteristics of the patients with SSc included in the discovery and replication sets*

	French cohort (n = 985)†	Italian cohort (n = 622)†
Female	84.3	90.7
Age, mean ± SD years	57.5 ± 13.9	57.6 ± 13.9
Disease duration, mean ± SD years	10.9 ± 8.2	12.1 ± 8.6
lcSSc	66.9	74.6
dcSSc	33.1	25.4
Anti–topo I+	26.9	32.7
ACA+	42.5	45.8
Fibrosing alveolitis seen on CT scan	40.4	35.0
At least 1 associated autoimmune disease	15.2	16.1
Sjögren's syndrome	8.4	6.2
Autoimmune thyroiditis	4.6	8.7
Primary biliary cirrhosis	2.3	1.4

* Except where indicated otherwise, values are the percent. SSc = systemic sclerosis; lcSSc = limited cutaneous SSc; dcSSc = diffuse cutaneous SSc; anti–topo I = anti–topoisomerase I antibody; ACA = anticentromere antibody; CT = computed tomography.
† Number of patients after exclusion of some patients from analysis for quality control purposes (see Patients and Methods).

and their putative antibody specificity, and for other antibodies if clinically relevant.

Genotyping and quantification of gene expression in peripheral blood mononuclear cells (PBMCs). We selected 4 *TREX1* SNPs (rs3135946, rs7626978, rs3135943, and rs11797) and 6 low-frequency *TNFAIP3* SNPs (rs9494883, rs72063345, rs5029939, rs2230926, rs117480515, and rs7749323) because of their reported associations with SLE (6–11) and/or because of their possible functional implications (10,11). Genotyping was performed as previously described (13). In addition, direct sequencing of the region downstream of *TNFAIP3* was performed in a random sample of 143 French SSc patients and 131 controls, to determine whether the polymorphic dinucleotide identified in SLE (rs117480515) was present (10). *TNFAIP3* gene expression by PBMCs was measured by TaqMan quantitative real-time polymerase chain reaction. (Details on the process for direct sequencing and for measurement of gene expression in PBMCs are available from the corresponding author upon request.)

Statistical analysis. Statistical analyses were conducted as previously described (13) and included power calculation and haplotype analysis. Subjects lacking genotyping data for at least one of the *TREX1* and *TNFAIP3* SNPs (n = 96 and n = 178, respectively) were excluded from the analyses. In addition, SSc patients with concomitant autoimmune diseases known to be associated with the *TREX1* and *TNFAIP3* loci (n = 18) were excluded prior to analysis in order to avoid bias due to a possible excess of risk alleles attributable to these patients, as previously described (13). The combined data on the 2 populations were analyzed by calculating the pooled ORs using a Cochran-Mantel-Haenszel test for stratified analysis. Ninety-five percent confidence intervals (95% CIs) were calculated. The Breslow-Day method was applied to calculate the homogeneity of ORs between the 2 cohorts.

RESULTS

Discovery set. The average genotyping call rate for the *TREX1* and *TNFAIP3* variants was >98% in both the SSc and the control samples. The 6 *TNFAIP3* variants and 3 of the *TREX1* variants (rs1197, rs3135946, and rs7626978) were in Hardy-Weinberg equilibrium in the control subjects in the discovery population. Allelic frequencies were found to be in good agreement with those reported in the European Caucasian population.

TREX1. No association was found between any of the 4 *TREX1* variants and SSc overall or specific subphenotypes of SSc (data available from the corresponding author upon request). The *TREX1* polymorphism rs3135943 described in SLE patients was not found in the French SSc and control populations, supporting the notion that it relates to a genetic mutation.

TNFAIP3. Direct sequencing of the region downstream of *TNFAIP3* revealed the presence of a single-base deletion at chromosome 6 position 138,271,732, followed by a T>A variation in 12 SSc patients and 7 control individuals (allelic frequency 4.23% and 2.69%, respectively; $P = 0.33$), all in a heterozygous state. The rs117480515 minor A allele that refers to the T>A variant at chromosome position 138,272,733 was detected in 100% of individuals carrying the T deletion. Conversely, the rs117480515 T allele was detected in all individuals who did not carry the deletion. We therefore considered that the rs117480515 A allele was a perfect proxy for this deletion, and that this result could be extended to our large genotyping cohort using rs117480515 as a tag SNP for the T deletion and the dinucleotide.

The case–control association study demonstrated an association of all *TNFAIP3* SNPs investigated with SSc (Table 2). When subphenotypes were considered and after Bonferroni correction for multiple testing, the most significant differences in allelic frequencies and ORs were observed for the subsets of SSc patients with diffuse cutaneous disease (dcSSc), with fibrosing alveolitis, and with a polyautoimmune phenotype (Table 2). The genotypes obtained for rs117480515 were in 100% agreement with those obtained by direct sequencing in individuals randomly selected to have both methods performed. Taking into account the observed association signal for *TNFAIP3*, we continued the investigation solely for this gene, and *TREX1* was not genotyped in the replication sample.

TNFAIP3 haplotype analysis and linkage disequilibrium (LD) relationship. Two common *TNFAIP3* haplotypes (frequency >1% in controls) were predicted from our study sample. Only the G-C-G-G-A-A haplotype, consisting of the minor alleles of each SNP, was found to be associated with SSc (4.46% in patients versus 1.82% in controls; $P = 2.63 \times 10^{-6}$). The other haplotype consisted of the major alleles of each SNP. Pairwise LD was calculated by analysis of the correlation (r^2) in the control population. Consistent with findings in the haplotype analysis, all SNPs demonstrated strong LD in the French Caucasian healthy control subjects (details available from the corresponding author upon request). Given that the *TNFAIP3* risk haplotype could be tagged by one single SNP, we subsequently only investigated SNP rs117480515 in the replication sample.

Replication set. The SNP rs117480515 was in Hardy-Weinberg equilibrium in the Italian control population. The average call rate for rs117480515 was >98% in both the SSc patients and the control subjects. The allelic association with SSc was replicated only in patients with the polyautoimmune phenotype (minor allele frequency 7.0%, versus 2.4% in controls; OR 3.02 [95% CI 1.53–5.94], $P = 0.002$). Associations shown in the discovery cohort were not replicated for other SSc subtypes, including dcSSc (minor allele frequency 3.8%; OR 1.58 [95% CI 0.78–3.20], $P = 0.139$) (other data available from the corresponding author upon request).

Table 2. Allelic distributions of the *TNFAIP3* SNPs in SSc patients and controls from the French discovery sample and results of the Cochran-Mantel-Haenszel test for the combined European Caucasian populations*

SNP, phenotype (n)	MAF, %	Allelic *P*	Adjusted *P*†	OR (95% CI)
French discovery sample				
rs9494883 G				
SSc (985)	5.6	4.04×10^{-8}	2.43×10^{-7}	2.60 (1.83–3.70)
SSc plus other autoimmune disease (150)	7.7	5.35×10^{-6}	3.21×10^{-5}	3.65 (2.17–6.12)
dcSSc (285)	7.1	1.80×10^{-7}	1.08×10^{-6}	3.32 (2.14–5.13)
SSc, anti–topo I+ (220)	5.9	1.20×10^{-4}	7.20×10^{-4}	2.76 (1.68–4.52)
lcSSc (575)	5.1	1.70×10^{-5}	1.02×10^{-4}	2.38 (1.60–3.53)
SSc, ACA+ (348)	4.9	5.99×10^{-4}	3.60×10^{-3}	2.26 (1.43–3.55)
SSc with fibrosing alveolitis (346)	6.2	2.29×10^{-6}	1.37×10^{-5}	2.91 (1.90–4.46)
Controls (1,011)	2.0	NA	NA	NA
rs72063345 C				
SSc (985)	4.7	6.56×10^{-7}	3.93×10^{-6}	2.56 (1.74–3.75)
SSc plus other autoimmune disease (150)	7.0	5.57×10^{-6}	3.34×10^{-5}	3.93 (2.27–6.79)
dcSSc (285)	6.0	1.63×10^{-6}	9.75×10^{-6}	3.31 (2.07–5.31)
SSc, anti–topo I+ (220)	4.8	8.89×10^{-4}	5.33×10^{-3}	2.62 (1.52–4.51)
lcSSc (575)	4.3	7.14×10^{-5}	4.29×10^{-4}	2.37 (1.55–3.64)
SSc, ACA+ (348)	4.0	2.51×10^{-3}	1.50×10^{-2}	2.19 (1.33–3.59)
SSc with fibrosing alveolitis (346)	5.6	1.73×10^{-6}	1.04×10^{-5}	3.12 (1.98–4.92)
Controls (1,011)	1.9	NA	NA	NA
rs5029939 G				
SSc (985)	6.3	4.76×10^{-8}	2.86×10^{-7}	2.38 (1.73–3.28)
SSc plus other autoimmune disease (150)	8.3	1.32×10^{-5}	7.92×10^{-5}	3.19 (1.96–5.20)
dcSSc (285)	7.9	2.44×10^{-7}	1.47×10^{-6}	3.01 (2.01–4.51)
SSc, anti–topo I+ (220)	6.8	1.32×10^{-4}	8.20×10^{-4}	2.57 (1.63–4.05)
lcSSc (575)	5.7	6.86×10^{-5}	4.11×10^{-4}	2.10 (1.46–3.03)
SSc, ACA+ (348)	5.5	1.58×10^{-3}	9.50×10^{-3}	2.03 (1.33–3.09)
SSc with fibrosing alveolitis (346)	6.8	7.92×10^{-6}	4.75×10^{-5}	2.56 (1.72–3.81)
Controls (1,011)	2.8	NA	NA	NA
rs2230926 T				
SSc (985)	6.2	1.62×10^{-7}	9.72×10^{-7}	2.30 (1.67–3.16)
SSc plus other autoimmune disease (150)	8.3	2.64×10^{-5}	1.58×10^{-4}	3.13 (1.93–5.10)
dcSSc (285)	7.9	5.08×10^{-7}	3.05×10^{-6}	2.96 (1.98–4.42)
SSc, anti–topo I+ (220)	6.8	1.55×10^{-4}	9.28×10^{-4}	2.52 (1.60–3.98)
lcSSc (575)	5.5	2.19×10^{-4}	1.31×10^{-3}	2.00 (1.39–2.88)
SSc, ACA+ (348)	5.5	1.74×10^{-3}	1.04×10^{-2}	1.99 (1.31–3.03)
SSc with fibrosing alveolitis (346)	6.8	9.31×10^{-6}	5.59×10^{-5}	2.51 (1.69–3.73)
Controls (1,011)	2.8	NA	NA	NA
rs117480515 A				
SSc (985)	4.3	4.25×10^{-6}	2.55×10^{-5}	2.46 (1.65–3.65)
SSc plus other autoimmune disease (150)	6.7	8.86×10^{-6}	5.32×10^{-5}	3.94 (2.25–6.90)
dcSSc (285)	5.8	1.93×10^{-6}	1.16×10^{-5}	3.39 (2.09–5.49)
SSc, anti–topo I+ (220)	4.8	6.16×10^{-4}	3.69×10^{-3}	2.77 (1.60–4.79)
lcSSc (575)	4.0	2.60×10^{-4}	1.56×10^{-3}	2.30 (1.48–3.58)
SSc, ACA+ (348)	3.7	4.73×10^{-3}	2.80×10^{-2}	2.14 (1.28–3.57)
SSc with fibrosing alveolitis (346)	5.5	2.00×10^{-6}	1.20×10^{-5}	3.20 (2.02–5.10)
Controls (1,011)	1.8	NA	NA	NA
rs7749323 A				
SSc (985)	4.6	7.85×10^{-7}	4.71×10^{-6}	2.57 (1.74–3.79)
SSc plus other autoimmune disease (150)	6.7	1.21×10^{-5}	7.24×10^{-5}	3.83 (2.19–6.70)
dcSSc (285)	6.3	1.74×10^{-7}	1.04×10^{-6}	3.62 (2.26–5.78)
SSc, anti–topo I+ (220)	5.0	3.84×10^{-4}	2.30×10^{-3}	2.82 (1.65–4.84)
lcSSc (575)	4.1	2.06×10^{-4}	1.24×10^{-3}	2.29 (1.48–3.54)
SSc, ACA+ (348)	4.0	2.19×10^{-3}	1.31×10^{-2}	2.25 (1.34–3.70)
SSc with fibrosing alveolitis (346)	5.6	1.36×10^{-6}	8.13×10^{-6}	3.20 (2.03–5.07)
Controls (1,011)	1.8	NA	NA	NA
Combined French and Italian populations‡				
rs117480515 A				
SSc (1,607)	2.97	1.00×10^{-5}	6.00×10^{-5}	1.99 (1.46–2.71)
SSc plus other autoimmune disease (250)	2.68	1.43×10^{-9}	8.58×10^{-9}	3.51 (2.28–5.41)
dcSSc (443)	2.70	6.51×10^{-7}	3.91×10^{-6}	2.62 (1.77–3.87)
SSc, anti–topo I+ (423)	2.39	2.90×10^{-3}	0.0174	1.90 (1.23–2.93)
lcSSc (1,039)	2.65	4.70×10^{-4}	2.82×10^{-3}	1.82 (1.29–2.57)
SSc, ACA+ (632)	2.58	4.50×10^{-4}	2.70×10^{-3}	1.96 (1.34–2.88)
SSc with fibrosing alveolitis (561)	2.62	3.57×10^{-5}	2.14×10^{-4}	2.19 (1.49–3.22)
Controls (1,504)	2.97	NA	NA	NA

* SNPs = single-nucleotide polymorphisms; SSc = systemic sclerosis; MAF = minor allele frequency; OR = odds ratio; 95% CI = 95% confidence interval; dcSSc = diffuse cutaneous SSc; anti–topo I = anti–topoisomerase I antibody; lcSSc = limited cutaneous SSc; ACA = anticentromere antibody; NA = not applicable.

† After Bonferroni correction.

‡ Overall allele frequencies for *TNFAIP3* rs117480515 and Cochran-Mantel-Haenszel test results.

Combined populations. Despite the different geographic origins of the 2 data sets, a test of combinability performed by the Breslow-Day method identified no significant differences between them. Pooled OR analysis with the Cochran-Mantel-Haenszel test showed that the rs117480515 A allele was strongly associated with susceptibility to SSc with associated autoimmune disease(s) (pooled OR 3.51, [95% CI 2.28–5.41], $\chi^2 = 36.6$) (Table 2). Furthermore, the combined analysis of each separate autoimmune disease subgroup revealed significant associations for all 3 of the most frequently reported autoimmune diseases, i.e., Sjögren's syndrome (OR 3.66 [95% CI 1.97–6.78], $P = 1.07 \times 10^{-5}$), autoimmune thyroid disease (OR 4.09 [95% CI 2.17–7.71], $P = 2.03 \times 10^{-6}$), and primary biliary cirrhosis (OR 5.07 [95% CI 1.76–14.6], $P = 0.00083$). Within-cohort analyses showed an association between the *TNFAIP3* rs117480515 A allele and SSc with other autoimmune disease (n = 250) as compared to SSc with no other documented autoimmune disease (n = 1,099) (OR 1.98 [95% CI 1.31–2.99], $P = 0.001$) (data not shown).

Significant but weaker associations were observed in the dcSSc subset and the SSc with fibrosing alveolitis subset. The within-cohort comparison between the dcSSc subset (n = 443) and the limited cutaneous SSc subset (n = 158) was not significant (OR 1.39 [95% CI 0.95–2.04], $P = 0.09$).

Functional consequences of the *TNFAIP3* rs117480515 polymorphism with regard to *TNFAIP3* mRNA expression. We then investigated the potential influence of the risk haplotype, tagged by SNP rs117480515, on *TNFAIP3* mRNA expression by PBMCs from 33 control subjects (none with the rs117480515 AA genotype, 2 with the TA genotype, and 31 with the TT genotype) and 38 SSc patients (none with the rs117480515 AA genotype, 3 with the TA genotype, and 35 with the TT genotype). *TNFAIP3* mRNA expression levels were significantly lower in individuals who carried the *TNFAIP3* rs117480515 A risk allele than in those who did not ($P = 0.02$) (Figure 1). No difference in *TNFAIP3* expression between patients and controls was observed (data not shown).

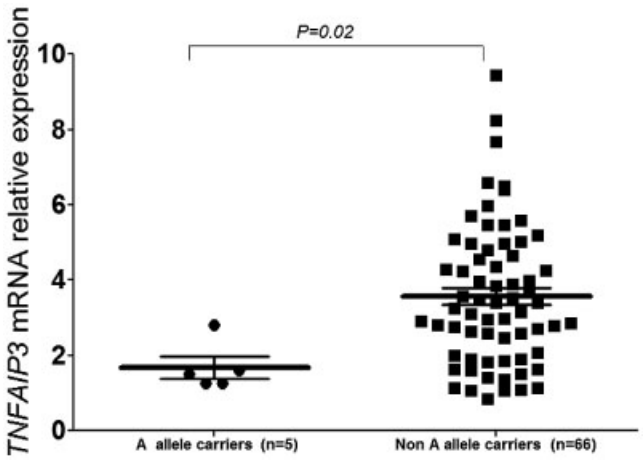

Figure 1. Influence of the *TNFAIP3* genotype on *TNFAIP3* mRNA expression by peripheral blood mononuclear cells, as measured by quantitative TaqMan polymerase chain reaction. Expression of *TNFAIP3* mRNA was significantly reduced in carriers of the rs117480515 A allele as compared with noncarriers of the A allele. Each symbol represents an individual subject; bars show the mean ± SEM.

DISCUSSION

Findings of the present association study provide strong evidence of a positive association between *TNFAIP3* low-frequency variants and the subset of SSc with concomitant autoimmune disorders in European Caucasian populations. This result was obtained in analyses of large cohorts and included a replication step confirming the strong association observed in the subset of SSc with polyautoimmunity. Within-cohort analyses further supported this association. It is noteworthy that the association with the *TNFAIP3* rs117480515 A allele was consistently observed when each associated autoimmune phenotype (Sjögren's syndrome, autoimmune thyroid disease, and primary biliary cirrhosis) was analyzed individually, further demonstrating that the *TNFAIP3* gene confers susceptibility to the polyautoimmune trait in general. This is of particular interest since the risk haplotype may therefore be considered a specific marker of polyautoimmunity.

We investigated a TT>A functional dinucleotide polymorphism in the downstream region of *TNFAIP3* (10). The presence of the T deletion composing this risk variant was identified recently by resequencing of the *TNFAIP3* locus in SLE patients, and was reported to be associated with reduced A20 protein expression (10). However, whether this deletion is present in patients with other autoimmune diseases and in healthy individuals was still unknown. Our results shed new light on this dinucleotide variant, since we found that the deletion 1) was present in 2.69% of control subjects, confirming that it is a low-frequency polymorphism and not a mutation, and 2) could be tagged by the contiguous SNP rs117480515. Furthermore, our haplotype analysis showed that the risk haplotype could be tagged by the rs117480515 A allele. When we addressed the question of the potential functional impact of this deletion on modulating *TNFAIP3* mRNA expression, we found

that carriers of the *TNFAIP3* rs117480515 A risk allele displayed decreased expression of *TNFAIP3* mRNA compared to noncarriers, consistent with the results observed in SLE (10). However, this first attempt to investigate the functional effect of the risk haplotype was limited by the small sample size and will need to be confirmed using a larger sample.

Recently reported converging data provide evidence of a central role of A20 in autoimmune pathways and suggest that defects in A20 expression might be involved in the development of autoimmune conditions. In SSc, gene expression profile analysis of endothelial cells derived from progenitor cells revealed a trend toward reduced *TNFAIP3* mRNA expression in SSc patients compared to healthy controls (14). In addition, reduced A20 protein expression was observed in skin tissue of SSc patients compared to controls. Furthermore, studies using conditional A20-knockout mouse models have shown that A20 deficiency in dendritic cells results in systemic B and T cell autoimmunity resembling an SLE phenotype, while selective A20 deficiency in myeloid cells triggers an erosive polyarthritis phenotype (15).

The results obtained in this case–control association study demonstrate that the investigated polymorphisms of the *TREX1* gene do not contribute to susceptibility to SSc or its subphenotypes. This lack of association strengthens the notion that while some genetic loci may confer susceptibility to several autoimmune phenotypes and support the concept of shared autoimmunity, other genes may be restricted to specific diseases. However, due to the low frequency of the *TREX1* variants investigated, our study was statistically underpowered to allow us to draw firm conclusions regarding the lack of association observed for *TREX1*. Studies of larger populations of cases and controls are thus needed to confirm this result. Furthermore, although we found similar allelic frequencies of the *TNFAIP3* SNP rs117480515 and used the Breslow-Day test to rule out genetic heterogeneity, the use of ancestry-informative markers would have further confirmed the absence of heterogeneity between French and Italian cohorts.

Our study establishes the *TNFAIP3* rs117480515 variant as a susceptibility factor in the subset of SSc with associated autoimmune disease, mostly represented by Sjögren's syndrome, autoimmune thyroiditis, and primary biliary cirrhosis. Moreover, the current results, obtained through the analysis of enriched phenotypes, also provide further evidence supporting the implication of rare variants in susceptibility to complex diseases. These findings should encourage investigators to perform more in-depth phenotyping of patients with autoimmune disease and to take into account polyautoimmunity in statistical analyses. Indeed, genetic factors such as *PTPN22*, *IRF5*, *STAT4*, and now *TNFAIP3* not only confer common genetic susceptibility to multiple autoimmune diseases, but also show potential as possible markers of individual and familial polyautoimmunity (1). Taken together, our data support the emerging role of A20 in various autoimmune diseases as highlighted in recent in vitro and in vivo studies (14,15). This molecule is progressively being identified as a crucial player in shared autoimmunity, and as a potential new therapeutic target that will hopefully lead to common treatment for different autoimmune diseases.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Allanore had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Koumakis, Dieudé, Cuomo, Matucci-Cerinic, Diot, Amoura, Sibilia, Carpentier, Guiducci, Meyer, Kahan, Boileau, Allanore.

Acquisition of data. Koumakis, Dieudé, Cohignac, Cuomo, Airò, Hachulla, Matucci-Cerinic, Caramaschi, Mouthon, Riccieri, Cracowski, Tiev, Francès, Amoura, Sibilia, Cosnes, Carpentier, Valentini, Manetti, Guiducci, Meyer, Kahan, Boileau, Chiocchia, Allanore.

Analysis and interpretation of data. Koumakis, Giraud, Dieudé, Tiev, Meyer, Kahan, Boileau, Chiocchia, Allanore.

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