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Concise report

FLT3 functional low-frequency variant rs76428106-C is associated with susceptibility to systemic sclerosis

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Abstract

Objectives. rs76428106-C, a low frequency polymorphism that affects the splicing of the *FLT3* gene, has recently been associated with several seropositive autoimmune diseases. Here, we aimed to evaluate the potential implication of rs76428106-C in the susceptibility to systemic sclerosis (SSc).

Methods. We analysed a total of 26 598 European ancestry individuals, 9063 SSc and 17 535 healthy controls, to test the association between *FLT3* rs76428106-C and SSc and its different subphenotypes. Genotype data of rs76428106 were obtained by imputation of already available genome-wide association study data and analysed by logistic regression analysis.

Results. In accordance with that observed in other autoimmune disorders, the *FLT3* rs76428106-C allele was significantly increased [P-value $= 2.03 \times 10^{-3}$, odds ratio (OR) $= 1.34$] in SSc patients compared with healthy controls. A similar risk effect was found when the main SSc clinical and serological subgroups were compared with controls. When comparing SSc patients with and without digital ulcers (DU), the rs76428106-C frequency was significantly increased in DU-positive SSc patients in comparison with DU-negative patients (P-value $= 0.036$, OR $= 2.16$).

Conclusion. This study is the first to report an association between rs76428176-C and SSc. Our results support the role of *FLT3* as a relevant gene in seropositive immune-mediated diseases and a potential biomarker for SSc microangiopathy.

Key words: SSc, low-frequency variant, seropositive immune-mediated diseases

BASIC SCIENCE

Rheumatology key messages

- Rare and low-frequency variants represent a potential source contributing to SSc missing heritability.
- The low-frequency *FLT3* rs76428106-C variant is associated with genetic predisposition to SSc.
- *FLT3* represents a common risk locus for seropositive immune-mediated inflammatory disorders.

Introduction

Systemic sclerosis (SSc) is an immune-mediated inflammatory disease (IMID) with low prevalence (1 in 10 000 people) characterized by skin and internal organs fibrosis, vasculopathy and anti-nuclear antibodies (ANA) synthesis [1]. Although SSc aetiology is not completely understood, both genetic and environmental factors are implicated in the development of the disease. In this regard, our current understanding of the genetic landscape of SSc has notably increased, mainly due to the development of large-scale genetic studies. Indeed, the most extensive genome-wide association study (GWAS) conducted in SSc to date identified 27 independent signals associated with susceptibility to this condition [2]. However, despite the great advances, it has been reported that genetic studies account only for the ∼20% of the estimated heritability [3]. Therefore, a substantial part of its genetic component remains unclear. It can be hypothesized that at least part of the SSc missing

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heritability could be explained by rare and low-frequency variants not usually tested in GWAS. In addition to the application of new technologies, such as whole-exome sequencing and whole-genome sequencing, the use of well-powered candidate gene association studies has proven to be useful in determining the contribution of less common variants to complex diseases including IMIDs [4].

Recently, a GWAS performed in autoimmune thyroid disease identified an association with a low-frequency variant, rs76428106-C [5]. In addition, this single-nucleotide polymorphism was also found to influence genetic susceptibility to other seropositive IMIDs, including systemic lupus erythematosus (SLE), rheumatoid factor (RF)/anti-citrullinated protein antibodies (ACPA)-positive rheumatoid arthritis (RA) and coeliac disease, but showed no association with seronegative disorders [5]. rs76428106 is located in the 15th intron of the Fms Related Receptor Tyrosine Kinase 3 (*FLT3*) gene, which encodes a tyrosine kinase receptor involved in multiple immune-related pathways [6]. Notably, rs76428106-C is a splicing quantitative trait loci associated to a pathobiological effect similar to that conferred by gain-of-function mutations located in the same gene, thus leading to an increase in blood monocyte count. In addition, a correlation between this risk allele and increased levels of its ligand [Fms Related Receptor Tyrosine Kinase 3 Ligand (FLT3L)] in serum have also been reported [5].

Given the biological relevance of this single-nucleotide polymorphism and its pleiotropic role in seropositive IMIDs, we aimed to analyse the potential implication of the *FLT3* functional variant rs76428106-C in the susceptibility to SSc and its main subphenotypes.

Methods

Study cohort

A total of 26 598 individuals with European ancestry, 9063 SSc patients and 17 535 ancestry-matched controls, were enrolled in the present study. All of them were included in the SSc GWAS published by our group [2]. Patients with SSc fulfilled the criteria of the 2013 ACR for this disease [7] and were stratified according to the extension of the fibrotic lesions (limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), with a more aggressive phenotype), by their autoantibody profile [anti-centromere positive (ACA+) and anti-topoisomerase positive (ATA+)], and by the presence of digital ulcers [digital ulcers positive (DU+) and digital ulcers negative (DU–)] as a sign of microangiopathy. The Consejo Superior de Investigaciones Científicas' ethics committee approved the study protocol, and written informed consent was obtained by study participants in accordance with the tenets of the Declaration of Helsinki.

Association analysis

Since the *FLT3* variant rs76428106 was not included in our previous SSc GWAS [2], rs76428106 genotype data were obtained by imputation of already available GWAS data [2] using the multi-ancestry TOPMed reference panel performed through the TOPMed imputation server (https://imputation.biodatacatalyst.nhlbi.nih.gov/). Imputation results with rsq ≤ 0.3 were removed from further analyses. Logistic regression analysis was carried out on allele dosages using a generalized linear model (glm) in R adjusting by five principal components and sex [2]. Briefly, allelic dosage from SSc patients (and its different stratifications) was compared with allelic dosage from healthy control samples. Odds ratios (ORs) were obtained from glm function in R and 95% CIs were calculated with confint function in R. Statistical significance threshold was declared at P-value ≤ 0.05.

Results

The distribution of the minor allele frequency of the rs76428106 *FLT3* variant in SSc patients, SSc subtypes and healthy controls is shown in Table 1. Of note, we observed a statistically significant increase [P-value $= 2.03 \times 10^{-3}$, OR (95% CI) = 1.34 (1.11, 1.63)] of the presence of the rs76428106-C allele in SSc patients (1.77%) compared with control individuals (1.49%).

Next, we assessed the potential association of the rs76428106-C allele with specific clinical or serological subtypes of SSc. As shown in Table 1, when allelic frequencies were compared between each SSc subphenotype and healthy controls, statistical significant differences were evident for all the subsets analysed [lcSSc: P-value $= 5.00 \times 10^{-3}$, OR (95% CI) = 1.36 (1.09, 1.70); dcSSc: P-value = 0.013, OR (95% CI) = 1.43 (1.07, 1.90); ACA+: P-value = 0.048, OR (95% CI) = 1.32 (1.00, 1.75); ATA+: P-value = 0.014, OR (95% CI) = 1.52 (1.08, 2.12)]. Consistent with this, no statistically significant differences between SSc patients with and without these clinical characteristics were observed (data not shown), suggesting that rs76428106-C is associated with the overall disease.

Finally, we studied the association between rs76428106-C polymorphism and the presence of DU in SSc patients. Noteworthy, as reported in Table 2, we found statistically significant differences in the frequencies of the variant between DU+ and DU– SSc patients [P-value = 0.036, OR (95% CI) = 2.16 (1.07, 4.36)]. However, no statistically significant differences were observed when DU+ and DU– SSc patients were compared with healthy controls [DU+: P-value = 0.224, OR (95% CI) = 1.41 (0.79, 2.4); DU–: P-value = 0.207, OR (95% CI) = 0.71 (0.41, 1.19)]. Hence, this association suggests that this variant could be potentially considered as a marker of microangiopathy in the disease context.

Discussion

Our data indicate, for the first time, that the *FLT3* rs76428106-C allele contributes to the genetic susceptibility to SSc. Notably, this same allele was positively correlated with FLT3L plasma levels in a previous work [5]. The FLT3/FLT3L axis regulates relevant processes, including

Table 1 Association analysis of rs76428106-C in SSc and its main clinical and serological subphenotypes

	Sample size	MAF (%)	*P*-value	OR	95% CI (L95, U95)
SSc	9063	1.77	2.03×10^{-3}	1.34	1.11, 1.63
Controls	17 535	1.49			
lcSSc	5665	1.78	5.00×10^{-3}	1.36	1.09, 1.70
dSSc	2529	1.87	1.33×10^{-2}	1.43	1.07, 1.90
ACA+	3331	1.72	4.82×10^{-2}	1.32	1.00, 1.75
ATA+	1746	1.96	1.39×10^{-2}	1.52	1.08, 2.12

ACA+: anti-centromere autoantibodies; ATA+: anti-topoisomerase autoantibodies; MAF: minor allele frequency; OR: odds ratio; L95, lower bound of the 95% CI; U95, upper bound of the 95% CI.

Table 2 Association analysis of rs76428106-C in SSc patients with and without digital ulcers (DU)

	Sample size	MAF (%)	*P*-value	OR	95% CI (L95, U95)
SSc-DU+	908	1.78	0.224	1.41	0.79, 2.4
SSc-DU–	1529	1.30	0.207	0.71	0.41, 1.19
Controls	17 535	1.49			
SSc-DU+ *vs* SSc-DU–			3.06×10^{-2}	2.16	1.07, 4.36

SSc-DU+: SSc patients with presence of digital ulcers; SSc-DU–: SSc patients without digital ulcers; MAF: minor allele frequency; OR: odds ratio; L95: lower bound of the 95% CI; U95: upper bound of the 95% CI.

angiogenesis, IL-10 systemic levels, and immune cell differentiation and proliferation. In addition, since FLT3L serum levels were increased in SSc patients exhibiting microangiopathic manifestations, it has recently been suggested as a microangiopathy biomarker [8]. Interestingly, our results show that this variant has a risk effect for the presence of DU in SSc patients. Therefore, taking together, these evidences point to a role of the FLT3/FLT3L axis in the development of vascular abnormalities in SSc patients. It is noteworthy that the FLT3/FLT3L signalling pathway downregulates IL-10 production. This signalling molecule plays an active role in SSc pathogenesis, as it has been demonstrated to dampen pro-inflammatory responses [9]. Moreover, it has also been reported that low levels of IL-10, together with TGF-β, promote the fibrotic process in the skin of SSc patients [10].

As mentioned, this axis is also involved in modulating immune cell balance; specifically, the activation of FLT3 plays a crucial role in proliferation and survival of haematopoietic and B cells progenitors, and acts as a key regulator of dendritic cells homeostasis and development [6]. In addition, it has been shown that rs76428106-C correlates with an increased monocyte count [5]. It is well-established that both innate and adaptive immune cells play a major role in SSc pathogenesis [3]. For instance, lymphocytic infiltration of affected tissues has been observed in the earlier stages of the disease, in which both B and T cells show an activated phenotype [11]. Monocytes from SSc patients increase their infiltration in fibrotic lesions, both in lung and skin, where abnormal M2 macrophage polarization aggravates the fibrotic process [12]. In addition, dendritic cells have also been described to play a relevant role in SSc, as they are widely implicated in key pathways, such as the IFN signature, and are highly infiltrated in skin lesions [11]. Notably, FLT3L has been found to promote the differentiation of common lymphoid progenitors into plasmacytoid dendritic cells [13], a major cellular source of type I IFN.

FLT3 belongs to the type III tyrosine-kinase receptors (RTKs) subfamily, a heterogeneous group of receptors involved in multiple pathways, developing an intricate network with some overlapping biological implications across different receptors [14]. Interestingly, other RTKs members, such as the soluble vascular endothelial growth factor receptor-2 (sVEGFR2) and the platelet-derived growth factor receptor (PDGFR), have previously been related with SSc. For instance, increased levels of sVEGFR2 have been reported in serum of SSc patients and in the related microangiopathy [15]. Additionally, PDGFR has been found to trigger fibrotic processes in SSc patients [16]. All these evidences support the role of RTKs, including FLT3, in the pathophysiology of the disease.

Our data reinforce the role of *FLT3* rs76428106-C as a novel common risk locus for seropositive IMIDs. Throughout the previous years, great effort has been made in the elucidation of the genetic component of these disorders, uncovering a significant number of susceptibility loci shared across them [3, 17]. Autoantibody production, a major common clinical manifestation, requires a close interaction between innate and adaptive immunity [17]. In this context, *FLT3* could be influencing this process through its effect in both myeloid and

lymphoid progenitors. Additionally, the observed association between *FLT3* and IMIDs also suggests an overlap with hematological malignancies, since rs76428106-C was also found to predispose individuals to acute myeloid leukaemia [5]. Indeed, immune-mediated inflammatory disease patients present an increased risk of these malignancies [18]. Moreover, it has been demonstrated that the analysis of overlapping genetic features can be useful in understanding the pathogenesis of both disorders [5]. Cancer-related genes are frequently associated with higher cell proliferation rates; considering the presence of an increased immune cell expansion in both haematological disorders and IMIDs, it is reasonable to hypothesize that *FLT3* might be involved in this excessive proliferative process.

So far, FLT3 is a target for several drugs (some of them already approved by the Food and Drug Administration), most of which are used to treat blood malignancies and other cancer types [6]. Considering that *FLT3* represents a common factor involved in the pathogenesis of different IMIDs, drugs targeting this protein could be potentially effective for these conditions. For instance, nintedanib inhibits several RTKs including FLT3 and is indicated for patients with chronic fibrosing interstitial lung diseases [19]. Lung fibrosis is one of the most severe clinical manifestations in SSc and has been associated with a higher mortality rate [1]. Interestingly, nintedanib has been shown to delay lung function decline in a Phase 3 clinical trial of SSc-interstitial lung disease patients [20] and has been recently approved by the Food and Drug Administration. Taken this evidence together, FLT3 could be a suitable candidate for drug repurposing in SSc. However, the blockage of this signalling pathway might have unexpected consequences when inhibiting the expansion of the already impaired regulatory T and B cells in SSc patients [9]. Therefore, functional experiments are required to shed light on how the immune balance is affected by these drugs.

In conclusion, concordantly with the already reported implication of the *FLT3* locus in different seropositive IMIDs, our results show a role of the rs76428106-C variant as a novel genetic risk factor contributing to the SSc susceptibility. Moreover, our findings also reinforce the hypothesis that *FLT3* and its ligand could be a microangiopathy biomarker in SSc.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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