The effect of body fat distribution on systemic sclerosis


Affiliations

1. Department of Cell Biology and Immunology, Institute of Parasitology and Biomedicine López-Neyra, CSIC, Granada, Spain.
2. Department of Internal Medicine, Valle de Hebrón Hospital, Barcelona, Spain.
3. Department of Internal Medicine, Hospital San Cecilio, Granada, Spain.
4. Department of Rheumatology, The University of Texas Health Science Center at Houston, Houston, Texas, USA.
5. Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy.
6. See supplementary notes.
7. Department of Rheumatology A, Hospital Cochin, Paris, Île-de-France, France.
8. NIHR Biomedical Research Centre, Guy's and Saint Thomas' NHS Foundation Trust and King's College, London, UK.
9. Center for Rheumatology, Royal Free and University College Medical School, London, UK.
10. Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands.
11. Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Solna, Sweden.
13. West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, China.
14. Departamento de Genética e Instituto de Biotecnología, Universidad de Granada, Granada, Spain.
15. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.

Correspondence to:

Lara Bossini-Castillo PhD

Departamento de Genética e Instituto de Biotecnología, Universidad de Granada, Centro
de Investigación Biomédica (CIBM), Parque Tecnológico Ciencias de la Salud, Avenida del Conocimiento, s/n, 18016, Armilla (Granada), Andalucía, Spain.

email: lbossinicastillo@ugr.es

Gonzalo Villanueva-Martin


e-mail: gvillanuevamartin@ipb.csic.es
Abstract

Obesity contributes to a chronic proinflammatory state, which is a known risk factor to develop immune-mediated diseases. However, its role in systemic sclerosis (SSc) remains to be elucidated. Therefore, we conducted a two sample mendelian randomization (2SMR) study to analyze the effect of three body fat distribution parameters in SSc.

As instrumental variables, we used the allele effects described for single nucleotide polymorphisms (SNPs) in different genome-wide association studies (GWAS) for SSc, body mass index (BMI), waist-to-hip ratio (WHR) and WHR adjusted for BMI (WHRadjBMI). We performed local (pHESS) and genome-wide (LDSC) genetic correlation analyses between each of the traits and SSc and we applied several MR methods (i.e. random-effects inverse variance-weight, MR-Egger regression, MR pleiotropy residual sum and outlier method and a multivariable model).

Our results showed no genetic correlation or causal relationship between any of these traits and SSc. Nevertheless, we observed a negative causal association between WHRadjBMI and SSc, which might be due to the effect of gastrointestinal complications suffered by the majority of SSc patients. In conclusion, reverse causality might be a specially difficult confounding factor to define the effect of obesity in the onset of SSc.
Introduction

Systemic sclerosis (SSc) is an immune mediated disease (IMD), characterized by abnormal immunological activation, vascular damage and fibrosis of the skin\(^1\). SSc represents a major challenge for clinicians as it has a deep impact on the life quality and life expectancy of the affected patients\(^1\). Recent efforts in the study of the genetic factors that contribute to the onset and progression of SSc, such as several large scale genetic association studies and genome wide association studies (GWAS)\(^2\), have contributed to identify risk alleles both in the Human Leukocyte Antigen (HLA) locus and outside this highly polymorphic region. Therefore, the number of relevant loci that have been firmly associated with this condition has remarkably increased over the last decade. Although the use of genetic risk factors to predict the risk of developing SSc was explored in a recent genomic risk score (GRS)\(^3\), the involvement of these genetic risk factors in the disease pathogenesis and the affected biological pathways have not been fully established yet\(^4\).

Despite the advances in the identification of the genetic factors contributing to the heritability of SSc, the complex nature of this disorder is an intrinsic obstacle to study the pathological mechanisms that lead to the disruption of the immune homeostasis and to the onset of fibrotic processes in affected individuals. Well-established environmental triggers for SSc are silica and solvents, which in extreme or long-term exposures are related to the disease development\(^5,6\). Moreover, demographic and clinical characteristics such as sex, age, ethnical origin, hormone levels, etc. have been pointed out as risk factors for SSc\(^5,7\). But the roles of life-style and environmental triggers in the manifestation and prognosis of SSc are still elusive.

Mendelian Randomization (MR) uses SNPs as instrumental variants (IVs) in order to determine if they are acting on a disease or outcome through a risk factor or exposure\(^8,9\). The principle of the methods is that alleles are randomly distributed during gametogenesis, as well as their presence pre-exists the disease. These genetic facts mimic the random distribution of clinical trials and take away the causality of the disease on the variable, reducing confounding factors\(^10\). For a genetic variant to be considered as a IV, it’s assumed that it is associated with the risk factor. However, an IV cannot be associated with any confounding factor related to the risk factor or the outcome neither directly nor indirectly. Additionally, the effects of the IV on the outcome should only be mediated by the exposure\(^8\). Therefore, only when genetic polymorphisms which are relevant, independent and have a restricted effect on the outcome can be considered as IVs. In a classical MR study, the allele effects on the outcome and exposure are obtained from the same individuals\(^8,9\). However, detailed information for multiple traits is difficult to obtain in a large population. Two-sample MR (2SMR) methods allow us to combine the estimations of the IV allele effects relying only on GWAS summary statistics for the outcome and for the exposure from independent studies. The implementation of these methods has improved the statistical power to detect causal associations between risk factors and disease, which has shown promising results in several conditions\(^11\).
Obesity-related diseases are becoming a public health issue in Western countries, since obesity rates are increasing due to unhealthy lifestyles. Obesity is defined by an excess of fat in the body and body fat distribution can be measured by a variety of methods, for instance body mass index (BMI) and waist to hip ratio (WHR). BMI is the most common body fat proxy and it is the gold-standard for obesity. BMI is measured as the body weight normalized by height square (kg/m²), and it is known that BMI > 25 kg/m² is associated with an increased risk to suffer from chronic diseases such as cardiovascular disease, type II diabetes or specific cancers. Nevertheless, BMI has certain limitations and anthropometric measures of abdominal obesity, such as WHR, seem to be better indicators of excessive fat mass. Since WHR measures both visceral and gluteal fat, it stands out among other anthropometric traits. If WHR is adjusted for BMI (WHRadjBMI), it is possible to obtain an anthropometric measure which is independent from the overall adiposity, and to combine the most standardized measure of obesity and the anthropometric measure that best captures the distribution of body fat. Taking advantage of the publicly available GWAS results, MR approaches have been successful in identifying risk factors for IMDs, such as obesity-related traits. The excess of fat has been associated with a low but persistent proinflammatory state that is believed to promote IMDs. However, in the case of SSc, the relationship between body fat distribution and SSc remains to be explored.

Consequently, in order to analyze the effect of nutritional-status on SSc risk, we applied the novel 2SMR methods on the largest GWAS of SSc patients with European ancestry and the biggest GWAS meta-analysis to date for fat distribution anthropometric measures to date.

Results

Making the most out of novel methodological strategies and the GWAS summary statistics of the largest SSc meta-analysis as an outcome and three obesity-related trait GWAS comprising thousands of European ancestry individuals as exposures, we studied for the first time the causal contribution of body fat distribution to the risk of suffering from SSc.

Genomic correlation. Only the HLA locus harbours local genetic correlation between SSc and body fat distribution

At a genomic scale, we observed a strong genome-wide correlation between BMI and WHR ($r_g = 0.59$, [95% CI -0.016 - 0.051]) and between WHR and WHRadjBMI ($r_g = 0.78$, [95% CI -0.01 - 0.03]), but not between WHRadjBMI and BMI ($r_g = -4.02 \times 10^{-2}$, [95% CI -0.016 - 0.049]), as previously described (Figure 2). However, our results showed no evidence of correlation between SSc and the three tested obesity-related traits (BMI $r_g = -0.039$ [95% CI -0.033 - 0.102]; WHR $r_g = -0.054$, [95% CI -0.035 - 0.106]; WHRadjBMI $r_g = -0.041$, [95% CI -0.04 - 0.122], all observed P > 0.05) (Figure 2).

Even when there is no correlation between traits at a genome-wide level, it is possible that the traits show local correlation at specific loci. To address this potential correlation, we performed a local genetic correlation analysis between BMI, WHR, WHRadjBMI and SSc.
The local correlation observed in these regions reached $r_g = 8.5 \times 10^{-4}$ and $r_g = 2.6 \times 10^{-4}$ (Supplementary Figure 1).

The analysis of the causal relationship between obesity-related traits and systemic sclerosis is limited by confounding factors.

Despite the limited genetic correlation found, we explored the possible causal relationship between body fat distribution and SSc. Considering the complex LD-patterns in the HLA-regions and the local genetic correlation found only in this locus, it was excluded from the following MR analyses. The available SSc dataset were powered enough to detect associations of 25% increased risk of SSc with BMI (98%), WHR (81%) and WHRadjBMI (91%) (Supplementary Table 1), considering an explained phenotypic variance of 2.5-5% and the complete set comprising 26,779 individuals (34.9% cases). We were confident about the statistical power estimated for the largest subsets of patients, for instance, females (BMI power = 78%, WHR power = 81% and WHRadjBMI = 86%), lcSSc (BMI power = 96%, WHR power = 73% and WHRadjBMI = 84%) and ACA+ (BMI power = 96%, WHR power = 73% and WHRadjBMI = 84%). However, the analyses for the less frequent patient groups, i.e. males (BMI power = 30%, WHR power = 9% and WHRadjBMI = 10%), dcSSc (BMI power = 30%, WHR power = 9% and WHRadjBMI = 10%) and ATA+ were clearly insufficient to identify true causal relationships (Supplementary Table 1).

As reported in Table 1 and Supplementary Table 2, classical MR methods showed no significant evidence of causality for BMI or WHR on SSc neither including only the index SNPs nor considering both the index SNPs and the secondary signals. The results for BMI under the random-effects IVW model showed a suggestive positive association with BMI, but this association did not reach statistical significance (OR under random-effects IVW = 1.15 [95% CI 0.67 - 1.98]) . Only a trend of negative association considering index and secondary signals was observed in the case of the random effects IVW model for WHR (Table 1). All the remaining models showed $P > 0.05$ and the ORs ranged 0.93 - 1.15 for BMI and 0.27 - 0.82 for WHR. In the case of WHRadjBMI (WHR after regressing out the effect of BMI), a negative association with SSc reached statistical significance in the three tested models (OR under random-effects IVW = 0.73 [95% CI 0.56 - 0.94], MR-Egger = 0.43 [95% CI 0.20-0.90], MR-PRESSO = 0.77 [95% CI 0.60-0.99]). These associations with WHRadjBMI remained negative in the analyses that included only index signals, but only the MR-Egger model was significant after multiple-testing correction (OR under MR-Egger = 0.69 [95% CI 0.51 - 0.93], (Supplementary Table 2).

Then, we carried out a sensitivity analysis, which implied the removal of SNPs associated with known obesity-related confounders (Supplementary Table 3), to address the effect of these confounders in the lack of significance for the BMI models and the negative relationships with WHR and WHRadjBMI. As shown in Table 2 and Supplementary Table 4, the confounder-free models did not change the observed negative relationship and none of them reached a significant result after FDR correction. Although we observed effect size heterogeneity for the different genetic variants (Supplementary Table 5), the analyses of the intercept parameter in the MR-Egger models did not reveal any signs of horizontal pleiotropy and the effects were not affected by the removal of the outlier SNPs identified by the MR-PRESSO algorithm (Tables 1-2, Supplementary Tables 2 and Supplementary Table 4). Furthermore, leave-one-out analyses did not highlight that these effects were influenced only by one variant (Supplementary Figure 2).
Finally, considering the significant associations observed for WHRadjBMI and the limitations of the univariate models to test for the combined influence of several exposures and to control for the effect of confounding factors, we decided to implement a MVMR model. This analysis allowed us to directly test the association of BMI and WHR with SSc controlling for the effects of both parameters at the same time. As expected, the results of these analyses showed an effect for WHR (MVMR OR 0.80 [95% CI 0.57-1.13]) that was similar to the previously identified effect for WHRadjBMI (Table 3). Nevertheless, no significant association of BMI with SSc was revealed (MVMR OR 1.03 [95% CI 0.79-1.33]) (Table 3). These findings might point towards a negative or inexistent effect of WHR in SSc and, if any, a very modest risk effect for BMI.

Considering the well-known clinical and genetic differences between the SSc subsets of patients 22, we explored subset-specific effects for the selected exposures. Several associations remained significant in the stratified analyses, especially in the largest and more powerful subsets, such as lcSSc (Supplementary Table 6). However, the direction and magnitude of the exposure effects were consistent in all the subsets (Supplementary Table 6), which suggested an uniform effect, if any, in all the patients. There were no significant differences between the models with and without the secondary signals (Supplementary Table 6). Moreover, taking into account the higher frequency of SSc in females (9 female: 1 male ratio) 7, we performed sex specific analyses too. In these analyses, we relied on female only and male only GWAS summary statistics for both SSc and the obesity-related risk factors. Once more, although the risk effect of BMI, WHR and WHRadjBMI seemed more evident in men, these effects did not reach statistical significance (Supplementary Table 6).
Discussion

This report addressed the risk effect of body fat distribution in SSc for the first time. We exploited to the maximum the availability of public GWAS summary statistics for both SSc and for anthropometric traits and the development of novel MR methods. We did not observe global genomic correlation between the outcome and any of the exposures. Moreover, local genetic correlation was only found in the HLA locus, a highly complex region. Different MR methods were then applied to identify possible causal relationships between the obesity traits and SSc. However, no significant risk causal effect of the exposures was found in this case.

Although our results do not support the causal relation between exposures and outcome, it should be noted that the statistical power of the SSc dataset is modest compared to similar studies performed to date in other IMDs, such as RA or IBD (Supplementary table 1). SSc is a rare IMD and, despite the recent advances, the recruitment of large patient cohorts remains challenging. Therefore, future efforts to enlarge the size or to complement the available SSc GWAS information might help to identify causal risk factors.

Additionally, the effect of confounders might be more severe in the case of SSc than in other IMDs. Gastrointestinal involvement (GI), which affects more than 70% of the SSc patients, hinders food ingestion and patients are mostly thin. Infact, weight loss has been used as one of the SSc diagnostic markers. This direct effect of the onset symptoms in the exposures is known as reverse causality, and it is a remarkably difficult confounding factor to control for. Reverse causality might be the cause behind both the lack of significant risk effects of BMI in SSc and the reported negative relationship between WHR and SSc, which becomes more evident when the effect of BMI is subtracted in the analysis of WHRadjBMI (Tables 1-2, Supplementary Tables 2-4).

Bad diet habits and obesity are associated with an increased risk to suffer from IMDs such as RA and IBD. Higher BMI has been associated with increased risk to Crohn’s disease (CD) and RA, but negative associations with BMI have been reported for ulcerative colitis (UC) and a recent study found reverse causality between WHR and RA. IMDs are often present as comorbidities and share altered molecular pathways, environmental triggers and genetic risk factors. Furthermore, the role of adipocytes in the activation of the immune system is prominent, especially due to the release of adipokines. Adipokines are molecules known to be involved in the "obesity-autoimmunity" relationship, such as lectins or cytokines, especially adiponectin, but also interleukins and tumour necrosis factor alpha (TNFα). Interestingly, patients with SSc and a high BMI have been shown to have higher lectin levels than healthy controls and it has been established that subcutaneous adipocytes can act as progenitor cells for fibroblasts. These fibroblasts may eventually transdifferentiate into myofibroblasts, activated profibrotic fibroblasts that are characteristic of the fibrotic lesions observed in SSc patients, and recent evidence has shown that the activation of adipocyte-derived mesenchymal cells from SSc skin biopsies to myofibroblasts is possible using soluble molecules present the skin microenvironment in SSc.
Considering all the above, in order to rule out the role of obesity as a risk factor for SSc, body-fat distribution measures from the patients before the onset of GI or BMI matched case-control sets would be very valuable resources.

Moreover, the negative association that is observed for WHR might be due to additional confounding factors that are inherent to SSc and that affect body fat distribution, for example, sex, lipid profiles, etc. Remarkably, WHR is different in women than in men and there is a clear sex-bias in SSc. Therefore, we hypothesized that there could be a sex-specific association and performed stratified analyses with the female and male cohorts separately. Our results showed significant causal associations with SSc only in the females, but considering the statistical power differences and the similarity between the effect sizes, the lack of significance for the male group may be likely due to the reduced sample size (Supplementary table 1). The key role of sample size as a limitation of our study to identify weak risk effects was also clear in other stratified analyses, as we found consistent ORs for all the tested clinical subtypes of SSc patients but the models reached statistical significance only in the largest subsets (Supplementary Table 6).

In conclusion, this study found no significant evidence that supported the role of body-fat distribution as causal risk factor for SSc using 2SMR methods. Nevertheless, the current GWAS have a limited statistical power to identify modest contributions to SSc risk and the intrinsic nature of the SSc clinical complications might be acting as potential constraints in this study. Consequently, further analyses will be needed to rule out the role of obesity in the onset of SSc.
Material and Methods

Instrumental variables

The study design of the 2SMR study for SSc and 3 obesity-related traits is summarized in Figure 1. The outcome instrumental variables (IV-outcome), i.e. the selected genetic variants and their effect sizes in SSc, were obtained from the largest SSc GWAS meta-analysis, which included 9,846 SSc patients and 18,333 healthy controls from 14 different cohorts with European ancestry. Additionally, SNP effect sizes after stratification by sex, serological and clinical subtype as reported elsewhere were also analyzed. Finally, we performed sex-specific analyses including only either the female or the male individuals from the different cohorts and following the previously described analysis framework.

In the case of the exposures, we obtained the IVs (IV-exposure) from a recent GWAS meta-analysis between the cohorts included in the Genetic Investigation of Anthropometric Traits consortium (GIANT) project and those recruited for the UK Biobank (UKBB) repository for different anthropometric measures. We only the summary statistics comprising individuals with the European ancestry, which included 806,810 individuals and 27,381,302 SNPs for BMI, a classical obesity parameter, and for two parameters that assess body fat distribution, WHR comprising 697,734 individuals and 27,376,273 SNPs and WHRadjBMI covering 694,649 individuals and 27,375,636 SNPs. None of the participants recruited in the SSc studies overlapped with the exposure GWASs to the best of our knowledge.

Genomic association analysis

Genetic correlation. To determine causality between obesity risk factors and SSc, we calculated the total genomic correlation between them. First, we performed an approximation implemented in the linkage disequilibrium regression score (LDSC) software. Then, to study the contribution of specific regions (pairwise local genetic correlation), we used the methods supported in the ρ-HESS software. Briefly, the ρ-HESS software splits the genome into 1,703 small regions through the chromosomes and uses LD matrices to create eigenvectors and to project the GWAS effect sizes. Then, local SNP-heritability per trait is calculated and, finally, genetic covariance between traits is estimated. We adjusted our significance thresholds for multiple testing, i.e. 1.1 x 10^{-3} (0.05/45) for LDSC and 2.9 x 10^{-5} (0.05/1,703) for ρ-HESS.

Mendelian randomization analysis. In order to assess if there was a causal relationship between body fat distribution and SSc or any of the stratified sets of patients, we performed a 2SMR study as implemented the R package “TwoSampleMR”. Considering the complex linkage disequilibrium (LD) patterns and the strong genetic associations described in the HLA locus SSc, the extended HLA region (chromosome 6: 20,000,000 - 40,000,000 bp) was excluded from the MR analyses in order to prevent biases.

The selected IVs were based on the original independent signal analysis reported by Pulit et al. Briefly, the independent signals from results from the inverse variance meta-analysis (P < 5 x 10^{-9}) were identified by LD-based clumping (r2 > 0.05 and ± 5Mb). Secondary signals were also defined by conditional analyses (P < 5 x 10^{-9}) and locus LD-clumping. We extracted the association estimates for these SNPs or the best available proxy (according to the LD
patterns observed in the UKBB cohort), which was present in the SSc dataset. The number of shared SNPs between SSc and the exposures reached 533, 247 and 262 for BMI, WHR, WHRadjBMI, respectively (Supplementary Table 7).

Three gold-standard 2SMR methods were selected. A random-effects inverse variance-weight (IVW) approach, which pools the effects of each IV and balances to zero the global pleiotropy by assuming the validity or invalidity of all the SNPs. A MR-Egger regression method, which is able to estimate causality even when all IVs are weak or invalid and to calculate horizontal pleiotropy. Although the previous methods are very robust for MR analysis, both of them have limitations to deal with outlier IVs. For that reason, we also applied the MR pleiotropy residual sum and outlier (MR-PRESSO) method. The MR-PRESSO algorithm detects outlier IVs that exert horizontal pleiotropy in a multi-instrument mendelian randomization analysis. Moreover, MR-PRESSO provides outlier-free causality estimates.

Additionally, to estimate the effect of the IVs controlling for their effect on other exposures, we performed a multivariable mendelian randomization analysis (MVMR) as implemented in the TwoSampleMR package. This analysis included a set of unique LD-clumped IV-exposures for both BMI and WHR, which were regressed against SSc together, weighting for the inverse variance of SSc for these IVs.

False Discovery Rate (FDR) Benjamini & Hochberg correction was applied, and we considered P < 0.05 as significant.

**Sensitivity analysis**

The statistical power of our analyses was calculated using the algorithm described by Brion et al for MR studies. Aiming to control for the effect of potential confounding factors, we removed from the MR analysis any the SNP with reported associations with known obesity-related confounding factors (Supplementary Table 3) as reported by the GWAS catalog, SNPnexus and ClinVar. We studied the contribution of each SNP to the observed effects by carrying out a leave-one-out sensitivity analysis, as implemented in the “TwoSampleMR” package. By these means, we observed that the exclusion of one SNP at a time did not affect the observed results.
References


36. Acosta-Herrera, M. *et al.* Comprehensive analysis of the major histocompatibility complex in systemic sclerosis identifies differential HLA associations by clinical and


FIGURE LEGENDS

**Figure 1.** Schematic representation of the study design. Selection of the instrumental variables for the outcome and the exposures, data harmonization and generation of different Mendelian Randomization models.

**Figure 2.** Pairwise global genetic correlation observed between the 3 obesity-related exposures and SSc. *=P>0.05 (suggestive for statistical significance); **=P> 0.00625 (Bonferroni corrected).

**Supplementary Figure 1.** Local genetic correlation, local genetic variance and local SNP-heritability between SSc and: i) BMI, ii) WHR and iii) WHRadjBMI.

**Supplementary Figure 2.** MR leave-one-out sensitivity analyses for: A) BMI, B) WHR and C) WHRadjBMI.

Data availability statement

Summary statistics of the SSc meta-GWAS is available through the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/downloads/summary-statistics)('Systemic Sclerosis' and/or 'Lopez-Isac/Martin' search terms). Obesity-related traits are available publicly through: https://zenodo.org/record/1251813#.YeAKN9uCGV5. All other data are contained in the article file and its supplementary information or available upon reasonable request to the corresponding authors.

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Collaborators

**International SSc Group:** P. Carreira, Department of Rheumatology, 12 de Octubre University Hospital, Madrid, Spain; I. Castellvi, Department of Rheumatology, Santa Creu i Sant Pau University Hospital, Barcelona, Spain; R. Ríos, Department of Internal Medicine, San Cecilio Clinic University Hospital, Granada, Spain; N. Ortego-Centeno, Department of Medicine, University of Granada, Granada, Spain; R. García Portales, Department of Rheumatology, Virgen de la Victoria Hospital, Málaga, Spain; A. Fernández-Nebro, Department of Rheumatology, Carlos Haya Hospital, Málaga, Spain; F. J. García-Hernández, Department of Internal Medicine, Virgen del Rocío Hospital, Sevilla, Spain; M. A. Aguirre, Department of Rheumatology, Reina Sofía/IMIBIC Hospital, Córdoba, Spain; B. Fernández-Gutiérrez, Department of Rheumatology, San Carlos Clinic Hospital, Madrid, Spain; L. Rodríguez-Rodríguez, Department of Rheumatology, San Carlos Clinic Hospital, Madrid, Spain; P. García de la Peña, Department of Rheumatology, Madrid Norte Sanchinarro Hospital, Madrid, Spain; E. Vicente, Department of Rheumatology, La Princesa Hospital,
Institute, Stockholm, Sweden; B. P. C. Koeleman, University Medical Center Utrecht, Utrecht, The Netherlands.

**Australian Scleroderma Interest Group (ASIG):** W. Stevens, St. Vincent's Hospital, Melbourne, Victoria, Australia; M. Nikpour, The University of Melbourne at St. Vincent's Hospital, Melbourne, Victoria, Australia; J. Zochling, Menzies Research Institute Tasmania, University of Tasmania, Hobart, TAS, Australia; J. Sahhar, Department Rheumatology, Monash Medical Centre, Melbourne, VIC, Australia; J. Roddy, Rheumatology, Royal Perth Hospital, Perth, WA, Australia; P. Nash, Research Unit, Sunshine Coast Rheumatology, Maroochydore, QLD, Australia; K. Tymms, Canberra Rheumatology, Canberra, ACT, Australia; M. Rischmueller, Department Rheumatology, The Queen Elizabeth Hospital, Woodville, SA, Australia; S. Lester, Department Rheumatology, The Queen Elizabeth Hospital, Woodville, SA, Australia.

**Contributors**

GVM: data analysis, manuscript drafting, revision and approval; MAH: data interpretation, manuscript revision and approval; MK: data interpretation, manuscript revision and approval; ELI: data interpretation, manuscript revision and approval; CPS: data acquisition, manuscript revision and approval; LB: data acquisition, manuscript revision and approval; YA: data acquisition, manuscript revision and approval; MAB: data acquisition, manuscript revision and approval; CF: data acquisition, manuscript revision and approval; CPD: data acquisition, manuscript revision and approval; TRDJ: data acquisition, manuscript revision and approval; MDM: data acquisition, manuscript revision and approval; XJ: study design, data interpretation, manuscript revision and approval; JM: study design, manuscript drafting, revision and approval; LBC: study design, manuscript drafting, revision and approval.

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**Competing interests**


**Ethics approval**

An ethical protocol was prepared, consensus was reached across all partners, academic and industrial, translated into all participants’ languages and approved by each of the local ethical committees of the clinical recruitment centers. The studies adhered to the standards set by
the International Conference on Harmonization and Good Clinical Practice (ICH-GCP), and to the ethical principles that have their origin in the Declaration of Helsinki (2013). The protection of the confidentiality of records that could identify the included subjects is ensured as defined by the EU Directive 2001/20/EC and the applicable national and international requirements relating to data protection in each participating country.
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<td>Method</td>
<td>SNPs</td>
<td>Beta</td>
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<td>0.39</td>
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</table>
**Total of individual per cohort**

- **Germany**: 10
- **Sweden**: 200
- **Italy**: 1000
- **Spain**: 1500

**Percent of esophagus involvement in all cohorts**

- **Esophagus involvement**
  - **Negative**: 33%
  - **Positive**: 67%

**Table**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Total of individual</th>
<th>Positive esophagus involvement</th>
<th>Negative esophagus involvement</th>
<th>Percent positive esophagus involvement</th>
<th>Percent negative esophagus involvement</th>
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<tr>
<td>Spain</td>
<td>1435</td>
<td>917</td>
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<tr>
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<td>170</td>
<td>24</td>
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<td>12.37%</td>
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<tr>
<td>Total</td>
<td>2245</td>
<td>1498</td>
<td>747</td>
<td>66.73%</td>
<td>33.27%</td>
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