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# Anti-RNPC3 (U11/U12) antibodies in systemic sclerosis are associated with moderate to severe gastrointestinal dysmotility.

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## Abstract

**Objective:** We examined the association of anti-RNPC3 antibodies in systemic sclerosis (scleroderma or SSc) patients with selected gastrointestinal (GI) tract complications.

**Methods:** Sera from SSc patients with or without severe GI dysfunction (total parenteral nutrition-dependence) from the Johns Hopkins Scleroderma Center were screened for anti-RNPC3 antibodies. We then examined anti-RNPC3-positive cases and negative SSc controls from the University of Pittsburgh and UMPC Scleroderma cohort to confirm our findings and to examine whether specific GI features were associated with anti-RNPC3 antibodies.

**Results:** In the discovery cohort, SSc patients with severe GI dysfunction (n=37) and without GI dysfunction (n=38) were screened for anti-RNPC3 antibodies. The former were more likely to have anti-RNPC3 antibodies (14% vs. 3%; p=0.11). In the Pittsburgh cohort, moderate to severe GI dysfunction (Medsger GI score ≥2) was present in 36% of anti-RNPC3 positive patients vs. 15% of anti-RNPC3 negative patients (p=<0.01). Anti-RNPC3 positive patients were more likely to be male (31% vs. 15%; p =0.04), black (18% vs. 6%; p =0.02), have esophageal dysmotility (93% vs. 62%; p <0.01), and interstitial lung disease (ILD, 77% vs. 35%; p <0.01). After adjusting for relevant covariates and potential confounders, moderate to severe GI disease was associated with anti-RNPC3 antibodies (OR= 3.8; 95%CI 1.0, 14.3), and ILD trended towards significance (OR= 2.8; 95%CI 1.0, 8.2).

**Conclusion:** Patients with SSc and anti-RNPC3 antibodies are more likely to be male, black and have moderate to severe GI disease and ILD. Further studies on larger patient cohorts may be helpful in further defining subsets of SSc patients at risk for severe GI involvement.

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Conflicts of interest:

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## INTRODUCTION

Gastrointestinal (GI) dysfunction is the most common internal complication of systemic sclerosis (SSc), affecting ≥ 90% of patients. The heterogeneity among patients with GI dysfunction is striking, as some patients have upper GI dysmotility, others lower GI dysmotility, and still others have both (1).

Small nuclear ribonucleoproteins (RNP) are recognized targets of the autoimmune response in SSc. While the protein portion of the complex is the most common target of the autoimmune response, distinct RNPs (e.g. U3RNP, U1RNP), are also well recognized. Recent reports (2, 3) suggest that an association between anti-RNPC3 (i.e. anti-U11/U12 RNP) antibodies and GI dysmotility in SSc may exist. However, one of these studies focused on a selected patient group (SSc patients with cancer), limiting the generalizability of the findings (2, 3). Furthermore, neither study assessed the association with distinct GI outcomes (2, 3).

In this study, we sought to determine whether anti-RNPC3 antibodies in SSc associate with severe GI dysmotility, and specific GI dysmotility complications. We initially compared patients on total parenteral nutrition (TPN) with asymptomatic patients from the Johns Hopkins Scleroderma Center and found that anti-RNPC3 antibodies are more prevalent among the former group. We then sought to confirm and expand this finding by comparing GI severity and examining the prevalence of specific GI complications in anti-RNPC3 positive and negative patients from the University of Pittsburgh and UMPC Scleroderma cohort.

## PATIENTS AND METHODS

### Patients.

The discovery cohort included all SSc patients with severe GI dysfunction (requiring TPN) and SSc patients without symptoms of GI dysfunction (modified Medsger severity score of 0) in the Johns Hopkins Scleroderma Center database (4). All patients meeting these GI criteria were included if they had both clinical data and banked serum, and met 2013 ACR/EULAR criteria, 1980 ACR criteria, or at least three of five features of CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome (5, 6, 7). Clinical charts of the cases and controls were reviewed to obtain details on SSc GI signs, symptoms, and severity, as well as to review all available objective GI tests. As this study was specifically focused on GI dysmotility, patients with gastric antral vascular ectasia (GAVE) were excluded. All study patients were evaluated as part of routine clinical care at the Johns Hopkins Scleroderma Center.

As our initial analysis suggested an association between anti-RNPC3 antibodies and severe SSc GI dysmotility, we subsequently performed a case-control study to confirm these findings using the University of Pittsburgh Scleroderma cohort. All anti-RNPC3 positive patients (cases) in the Pittsburgh database, first evaluated between 1980 and 2015, were identified and then matched to the next three consecutive anti-RNPC3 negative SSc patients (controls) evaluated in clinic. The most extreme points in the Pittsburgh database were used

to capture phenotype. GI severity (moderate to severe; Medsger severity score of ≥2) and the prevalence of specific GI characteristics were compared between groups. All cases and controls met the SSc classification criteria described above.

Written informed consent was obtained from all patients at both sites. The Johns Hopkins University and University of Pittsburgh Institutional Review Boards approved this study.

## Clinical Phenotyping.

**The Johns Hopkins Scleroderma Center (discovery cohort).—**The Center's database captures demographic and detailed clinical data at first encounter, and every 6 months thereafter at follow-up visits. Disease duration was defined from the time of the first symptom (Raynaud's or non-Raynaud's) that was attributed to SSc by the treating physician, to the date of serum sample collection (sample tested for anti-RNPC3 antibodies). Patients are classified as having diffuse or limited SSc based on the extent of skin involvement. Cutaneous thickening proximal to the elbows and knees or involving the trunk at any time during the illness is considered diffuse SSc, and thickening always only distal to the elbows and knees is considered limited SSc. Objective evidence of severe GI dysmotility was determined by physician documentation in the clinical notes and/or the presence of one or more of the following: (1) esophageal dysmotility as determined by hypomotility or abnormal lower esophageal sphincter on esophageal manometry, esophageal dilation on EGD, dilation of the esophagus on fluoroesophagopharyngogram, esophageal transit delay on scintigraphy-based whole gut transit study, patulous esophagus on CT chest, or esophageal hypomotility identified on barium swallow; (2) Gastroparesis as determined by delayed gastric emptying on a scintigraphy-based gastric emptying study or whole gut transit study; (3) Small bowel dysmotility as determined by distention, dilation, pseudo obstruction, or pneumatosis intestinalis and/or air fluid levels on an abdominal radiograph or CT, dilation and/or markedly delayed small bowel transit on upper GI small bowel series, hydrogen breath test documenting small bowel bacterial overgrowth, small bowel follow-through confirming the presence of dilated intestinal loops with features of pseudo obstruction, or hypomotility in the small bowel as determined by scintigraphy-based whole gut transit study; (4) Colonic dysmotility as defined by abnormal motility on a sitz marker study, abdominal radiograph or CT demonstrating dilated loops of colon, or a barium enema with dilated air-filled colon, or colonic hypomotility as determined by scintigraphy-based whole gut transit study. Cardiac involvement was defined by a score of 1 or greater on the Medsger severity scale (4, 8). Skin involvement was scored with the maximum modified Rodnan skin score (MRSS [range 0–51]). Skeletal myopathy (myopathy) was considered present when patients had an abnormal creatinine phosphokinase (CPK) and muscle weakness and/or abnormal electromyography (EMG), magnetic resonance imaging (MRI), or muscle biopsy (8). Pulmonary function was determined based on findings on pulmonary function tests (PFTs) (minimum forced vital capacity [FVC] and minimum single breath diffusing capacity for carbon monoxide [DLCO] at any visit) (9, 10). Estimated right ventricular systolic pressure (RVSP) was measured by echocardiogram and obtained as part of routine clinical screening for pulmonary hypertension; the maximum value at any visit was used for analysis. Sicca symptoms were defined as previously described (11). Renal crisis was confirmed by renal biopsy in the context of an acute symptomatic increase in

blood pressure. All antibody data, outside of anti-RNPC3 status, was obtained from the EUROIMMUN immunoblot assay (Systemic Sclerosis Profile) which was performed on the baseline serum sample.

**University of Pittsburgh Scleroderma Center (confirmatory cohort).—**The Pittsburgh database contains demographic and clinical data, including SSc subtype, organ system symptoms, and objective testing at baseline. The approach to clinical phenotyping of the Pittsburgh patients was consistent with those described for the Johns Hopkins Scleroderma cohort, with the exception of small differences in the GI, ILD, and myopathy outcomes and calculation of disease duration. In this cohort "moderate to severe GI dysmotility" was defined by distal esophageal aperistalsis, antibiotics for bacterial overgrowth, the presence of malabsorption syndrome, episodes of pseudo-obstruction, or the requirement for hyperalimentation (Medsger GI severity score ≥ 2). Given the heterogeneity of SSc GI findings, specific upper and lower GI outcomes were also recorded. The outcomes collected in the Pittsburgh database include: [1] patient-reported symptoms of acid reflux ("heartburn"); [2] patient-reported symptoms of distal dysphagia for solid foods; [3] esophageal dysmotility on imaging; [4] hypomotility or the presence of small bowel dilatation on radiographic studies; [5] the initiation of antibiotics for small intestinal bacterial overgrowth or physician-judged malabsorption syndrome and/or [6] the presence of dilated loops of bowel on a radiographic study. The presence of ILD was defined by radiographic evidence of bibasilar fibrosis and/or FVC <70% without obstructive findings. FVC was also analyzed as a longitudinal variable, using the lowest recorded percent predicted FVC in each patient. Muscle involvement was defined by the presence of proximal muscle weakness on physical examination (Medsger score ≥ 1), and CK of >2 times the upper limit, and/or abnormal EMG, MRI, or muscle biopsy consistent with myopathy. Disease duration was calculated from the date of any first symptom to the time of the first visit.

### Anti-RNPC3 antibody assay.

In the discovery cohort, anti-RNPC3 antibodies were assayed by immunoprecipitation of $^{35}$S-methionine labeled protein generated by in vitro transcription and translation (IVTT) from cDNA encoding full length RNPC3 (Origene Technologies) (12). These antibodies were assayed in the Pittsburgh cohort using serum samples from the first visit as described previously (3). As the assays used by the two Centers were different, all Pittsburgh cases with an available banked serum sample (n=41/49) and a sample of 15 randomly selected Pittsburgh controls were re-assayed by immunoprecipitation using RNPC3 generated by IVTT at the Johns Hopkins site. Anti-RNPC3 antibodies were confirmed in 39/41 of the Pittsburgh cases, and were not present in any of the 15 controls tested. Pittsburgh cases in which the anti-RNPC3 antibody status was not confirmed at Johns Hopkins (n=2) or sera were unavailable for re-assay (n=8), and their corresponding controls, were excluded from the analysis.

### Statistical analysis.

In the first case-control analysis (Johns Hopkins), the outcome of interest was severe GI dysmotility (dependent variable) defined as severe GI dysmotility (e.g. requiring TPN;

Medsger GI severity score of 4) versus no symptoms of GI dysmotility (modified Medsger GI severity score of 0). In the confirmatory case-control analysis, we examined the association between anti-RNPC3 positive status (dependent variable) and the severity of SSc GI dysmotility (GI Medsger severity score ≥ 2), specific GI complications, and other non-GI clinical features. Pearson correlation tests for parametric continuous variables and Spearman correlation tests for non-parametric continuous variables were conducted. Evaluation for associations between dichotomous variables was done using Chi-square or Fisher exact tests. Comparisons of continuous variables were performed using Student's t-test (parametric data) and the Wilcoxon signed-rank test (nonparametric data) of matched samples. The association of GI severity and specific GI complications with anti-RNPC3 positive status was evaluated using conditional logistic regression models consisting of the anti-RNPC3 status indicator and potential covariates. We then constructed models to explore the association between moderate to severe GI disease and anti-RNPC3 antibodies in SSc and included the following: (1) unadjusted model with only the GI severity variable; (2) a simple adjusted conditional logistic model adjusting for age, race, and GI severity; and (3) an adjusted conditional logistic model using covariates included by backwards selection. For the backwards elimination, we compared the p-value with a preselected significance level, 0.2. If it was statistically non-significant, then the variable got dropped. The AIC and likelihood ratio tests were used for selecting the best-fitted model. Statistical computing was conducted by Stata 14.0 (StataCorp, College Station, TX, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), with statistical significance defined as a 2-sided p-value <0.05.

## RESULTS

### Discovery study evaluating the association between severe SSc GI dysmotility and anti-RNPC3 antibodies in the Johns Hopkins cohort.

SSc sera from 37 patients with severe GI dysmotility (requiring TPN) and 38 patients without symptoms and/or objective findings of GI dysmotility (modified Medsger GI severity score of 0) were assayed for antibodies to RNPC3. All cases and none of the controls were confirmed to have severe GI dysmotility requiring TPN documented in the physician notes. In addition, 78% (29/37) of cases had objective testing reports available for review, also supporting the presence of GI dysmotility. The symptoms associated with TPN initiation were progressive weight loss, dysphagia, malabsorption, and/or recurrent pseudo obstruction, which occurred in the context of severe GI dysmotility.

Table 1 summarizes the clinical features of these two groups. Anti-RNPC3 antibodies were more frequently detected in patients with severe GI dysmotility compared to controls (14% vs. 3%; p = 0.11). Patients in the severe GI group were significantly more likely to be male (38% vs. 16%; p=0.031), black (43% vs. 13%; p=<0.01), have diffuse disease (65% vs. 34%; p =<0.01), myopathy (24% vs. 5%; p = 0.05), and anti-U3RNP antibodies (12% vs. 0%; p = 0.05). Severe GI patients were significantly less likely to have anti-RNA pol 3 antibodies (3% vs. 25%; p = 0.01). Two patients in the severe GI group were double-positive for antibodies, having both anti-RNPC3 antibodies, and antibodies to either Ro52 or PM-Scl.

### Confirmatory study defining specific GI characteristics associated with anti-RNPC3 positive SSc patients in the Pittsburgh cohort.

Since the number of anti-RNPC3 antibody positive patients in the Johns Hopkins discovery study was small, but anti-RNPC3 antibodies were over four times more frequent than expected in the severe GI group, we pursued additional analyses to understand this association using the current Pittsburgh Scleroderma cohort. This cohort is larger than the original published cohort that demonstrated anti-RNPC3 (anti-U11/U12 RNP) as an important specificity in SSc (3), and included 39 anti-RNPC3 antibody positive cases and their 3:1 matched anti-RNPC3 negative controls (n=117) (see Methods section).

Age, disease duration, and disease subtype were not significantly different between anti-RNPC3 antibody positive and negative patients in the Pittsburgh cohort (Table 2). Anti-RNPC3 antibody positive patients were more likely to be black (18% vs. 6%; p = 0.02) and male (31% vs. 15%; p = 0.04). Likewise, they were more likely to have moderate to severe GI dysfunction (36% vs. 15%; p = <0.01). Twenty-four of the 31 patients (77%) with significant GI dysmotility (Medsger score of 2 or greater) had confirmatory objective testing available in the database. Anti-RNPC3 antibody positive patients were also more likely to have ILD (77% vs. 35%; p <0.01), and the FVC was significantly lower in anti-RNPC3 positive cases compared to controls (67% predicted vs. 76% predicted; p=0.03). The distribution of other clinical features did not differ between groups (Table 2).

We then examined the specific GI features associated with anti-RNPC3 antibodies in the Pittsburgh cohort (Supplemental Table). Anti-RNPC3 positive patients were significantly more likely to have esophageal dysmotility (92.6% vs. 62.3%; p<0.01), as defined by evidence of esophageal dysmotility on imaging or manometry, although the presence of distal dysphagia and heartburn were not significantly different between groups. There was no significant difference between the presence of other features of GI disease and the presence of anti-RNPC3 antibodies. Although the numbers of patients studied are small, all comparisons favored higher frequencies of objective small intestinal involvement in anti-RNPC3 antibody positive patients.

We then sought to determine whether the association between clinical variables and anti-RNPC3 antibodies from the bivariate analysis remained after adjusting for relevant covariates and potential confounders. In the unadjusted model (Model 1), moderate to severe GI disease was associated with a 3.8 times increased odds of having anti-RNPC3 antibodies (95%CI 1.5, 9.8). In the simple adjusted model (Model 2), which was adjusted for age, race, and GI severity covariates, moderate to severe GI disease was again associated with a 3.8 times increased odds of having anti-RNPC3 antibodies (95%CI 1.4, 10.0). However there was no significant association for age (OR=1.0; 95%CI 0.95, 1.0) or black race (OR=2.4; 95%CI 0.7, 8.5). In the fully adjusted model (Model 3; covariates selected by backwards selection), patients with moderate to severe GI disease continued to have a 3.8 increased risk of having anti-RNPC3 antibodies (95%CI: 1.0, 14.3) (Table 3). There was no detectable change in the risk of having anti-RNPC3 antibodies per year increase in age at 1$^{st}$ visit (OR=1.0; 95% CI: 0.94–1.01), or with black race (OR=5.6; 95%CI 0.6, 48.7), diffuse cutaneous disease (OR=1.9; 95%CI: 0.8, 4.8), or myopathy (OR=0.1; 95%CI 0.0, 0.8). An association with ILD trended towards significance (OR=2.8; 95%CI 1.0, 8.2).

## DISCUSSION

This study evaluated the association between the severity of GI dysmotility and anti-RNPC3 antibodies, and whether specific GI complications were more frequent in this autoantibody subset. In our initial discovery analysis, we screened for anti-RNPC3 antibodies and compared their prevalence among SSc patients with severe GI dysmotility (TPN dependence) and those without symptoms of GI dysmotility. We found that the frequency of anti-RNPC3 antibodies is increased in the severe GI SSc population compared with SSc patients without symptoms of GI dysmotility (14% vs. 3%), consistent with findings in the published literature (3). We then further explored the association between anti-RNPC3 antibodies and GI dysmotility using a second cohort (Pittsburgh), and demonstrated an association between these antibodies and the presence of moderate to severe GI disease and esophageal dysmotility confirmed by objective testing. These data suggest that anti-RNPC3 antibodies are a marker of clinically important GI dysmotility in SSc.

The association between anti-RNPC3 antibodies and both pulmonary fibrosis and esophageal dysmotility in SSc is interesting. High rates of ILD are reported in association with anti-RNPC3 antibodies in SSc, with anti-RNPC3 antibody positive patients having an estimated 70% prevalence of ILD (3). In addition, recent studies suggest that microaspiration in SSc patients with uncontrolled reflux could contribute to the development of pulmonary fibrosis (13–15). Anti-RNPC3 antibodies may identify a specific subset of patients at higher risk for microaspiration that would benefit from more aggressive GERD management.

Our study confirms and extends observations made in two earlier reports. These earlier studies were limited by single-center assessments (3), cancer bias in sample selection (2), and did not examine GI complications as the primary outcome measure (2, 3). The current study utilized the power of two large carefully phenotyped SSc observational cohorts to examine the association between anti-RNPC3 antibody positive status, GI tract involvement severity, and specific GI complications. Narcotic use prior to the initiation of TPN was not widely available across the cohort and thus limited our analysis in this regard. Our study was limited by its retrospective design, as not all patients had complete GI assessments with objective testing (usually due to lack of symptoms warranting clinical testing). Prospective longitudinal data are needed to confirm our findings.

## CONCLUSIONS

An association between anti-RNPC3 antibodies, GI tract involvement severity and specific GI dysmotility characteristics exist in SSc patients. This occurs alongside a very high rate of ILD. Further studies examining the utilization of anti-RNPC3 antibodies as biomarkers for risk stratification of GI dysmotility in patients, and detailed studies of association of GI severity and ILD in SSc patients should be performed.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## SIGNIFICANCE AND INNOVATIONS

- Anti-RNPC3 antibody positive patients are significantly more likely to have moderate to severe GI dysfunction, even after adjustment for relevant covariates.
- Esophageal dysmotility is more prevalent among anti-RNPC3 positive patients with SSc.
- Antibody status may inform GI risk stratification and associate with specific GI clinical complications in patients with SSc.
- Finally, very high rates of interstitial lung disease in anti-RNPC3 antibody positive patients are further confirmed in this study.

**Table 1:**

Demographic disease and autoantibody characteristics of the 37 systemic sclerosis patients with severe GI dysfunction (TPN dependence), and the 38 patients without symptoms of GI dysfunction in the discovery cohort (Johns Hopkins Scleroderma Center).

| Variable | Severe GI (n=37) | No GI (n=38) | P-value |
|---|---|---|---|
| Age, year (range 23–89) | 53.7 ± 15.3 | 53.9 ± 16.1 | 0.95 |
| **Male, %** | **37.8** | **15.8** | **0.03** |
| **Race, %** | | | |
| **African American** | **43.2** | **13.2** | **<0.01** |
| Ever smoker, % | 46.0 | 52.6 | 0.56 |
| **Cutaneous subtype, diffuse,^ %** | **64.9** | **34.2** | **<0.01** |
| Disease duration, yrs (range 0.4–46) *† | 8.6 (4–20) | 6.8 (3–16) | 0.70 |
| Rodnan skin score, modified (range 0–47)^† | 12.0 (4–30) | 5.5 (3–22) | 0.06 |
| Raynaud's (> 1)^, % | 67.6 | 50.0 | 0.12 |
| Lung involvement (≥1)^, % | 86.5 | 68.4 | 0.10 |
| Cardiac involvement (≥1)^, % | 43.2 | 26.3 | 0.12 |
| **Skeletal myopathy,^ % (n)** | **35.1 (13/37)** | **13.2(5/38)** | **0.03** |
| Tendon friction rub,^% (n) | 20.0 (35) | 16.7 (36) | 0.72 |
| Renal crisis,^% (n) | 8.8 (3/34) | 0.0 (0/38) | 0.10 |
| Sicca complex,^% | 59.5 | 39.5 | 0.08 |
| Pulmonary function | | | |
| Minimum FVC, %predicted (n) | 62.8 ± 21.4 (n=35) | 72.2 ± 23.1 (n=35) | 0.08 |
| Minimum DLCO, %predicted (n) | 60.1 ± 29.6 (n=34) | 67.7 ± 29.0 (n=34) | 0.29 |
| RVSP, mmHg† (IQR) (n) | 39.3 (35–44) (n=34) | 35.0 (28–50) (n=27) | 0.38 |
| Autoantibodies, % | | | |
| Anti-topoisomerase-1 | 11.8 (4/34) | 19.4 (7/36) | 0.52 |
| Anti-centromere | 17.7 (6/34) (5/18) | 30.6 (11/36) | 0.27 |
| Anti-RNA polymerase 3 | 2.9 (1/34) | 25.0 (9/36) | **0.01** |
| Anti-Ro52 | 29.4 (10/34) | 33.3 (12/36) | 0.80 |
| Anti-Ku | 2.9 (1/34) | 5.6 (2/36) | 1.00 |
| Anti-PmScl | 0.0 (0/34) | 5.6 (2/36) | 0.49 |
| Anti-ThTo | 2.9 (1/34) | 8.3 (3/36) | 0.62 |
| Anti-U3RNP | 11.8 (4/34) | 0.0 (0/36) | **0.05** |
| Anti-RNPC3 | 13.5 (5/37) | 2.6 (1/38) | 0.11 |

GI = gastrointestinal;

*Disease duration from any first symptom to the date of the serum sample collection; n=number;

†Median (IQR);

^max ever Medsger severity score in the database; FVC and DLCO are represented by the minimum values ever recorded; n = patients with available data

**Table 2.**

Clinical characteristics of Anti-RNPC3 positive and negative patients in the confirmation study (University of Pittsburgh Scleroderma Cohort)

| Variable | Anti-RNPC3 positive (n=39) | Anti-RNPC3 negative (n=117) | P-value |
|---|---|---|---|
| **Age, year** | **47.1 ± 13.4** | **52.4 ± 14.8** | **0.05** |
| **Male, %** | **31** | **15** | **0.04** |
| **Black race, %** | **18** | **6** | **0.02** |
| Cutaneous subtype, diffuse,^ % | 56 | 44 | 0.17 |
| Disease duration, yrs *+ | 3.1 (2.0, 5.7) | 4.4 (1.1, 14.0)'d ju | 0.91 |
| **Moderate to severe GI disease, %** | **36** | **15** | **<0.01** |
| **Interstitial lung disease, %** | **77** | **35** | **<0.01** |
| Skeletal myopathy,^ % | 5 | 14 | 0.15 |
| Minimum FVC, %predicted (n) | 66.8 ± 22.2 (38) | 76.3 ± 21.0 (82) | 0.03 |

n= number; SD = standard deviation; IQR = interquartile range; GI = gastrointestinal; FVC = forced vital capacity; yrs = years;

+disease duration from any first symptom to first visit

**Table 3.**

Statistical models evaluating the association between anti-RNPC3 positive systemic sclerosis patients and the presence of moderate to severe GI disease in the confirmatory study (University of Pittsburgh Scleroderma Center cohort)

| | Unadjusted Conditional Logistic Model 1 | | Adjusted[1] Conditional Logistic for Age and Race Model 2 | | Adjusted[2] Conditional Logistic for significant covariates Model 3 | |
|---|---|---|---|---|---|---|
| **Characteristic** | **Odds Ratio** | **95% Confidence Interval** | **Odds Ratio** | **95% Confidence Interval** | **Odds Ratio** | **95% Confidence Interval** |
| Significant GI dysfunction | | | | | | |
| Medsger GI score <2 | 1.00 | | 1.00 | | 1.00 | |
| Medsger GI score ≥2 | 3.84 | 1.50 – 9.83 | 3.79 | 1.43 – 10.02 | 3.81 | 1.02 – 14.28 |
| Race | | | | | | |
| Non-African American | 1.00 | | 1.00 | | 1.00 | |
| African American | 3.58 | 1.11 – 11.48 | 2.36 | 0.65 – 8.54 | 5.59 | 0.64 – 48.7 |
| Diffuse cutaneous disease | 1.61 | 0.80 – 3.25 | - | - | 1.90 | 0.75 – 4.78 |
| Interstitial lung disease (ILD) | 5.85 | 2.33 – 14.65 | - | - | 2.79 | 0.95 – 8.19 |
| Myopathy | 0.36 | 0.08 – 1.61 | - | - | 0.07 | 0.01 – 0.75 |
| Age at Visit 1 | 0.97 | 0.95 – 1.00 | 0.97 | 0.95 – 1.00 | 0.97 | 0.94 – 1.01 |

GI = gastrointestinal;

*1.* Adjusted conditional logistic regression model, adjusted for age and race;

*2.* Adjusted conditional logistic regression model, adjusted for age and race, ILD, Diffuse cutaneous disease, Myopathy. Those covariates were selected by backwards selection.