Autoantibody Predictors of Gastrointestinal Symptoms in Systemic Sclerosis

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Abstract

Objectives: To assess the prevalence and burden of systemic sclerosis- (SSc) related gastrointestinal dysfunction (SSc-GI) and to evaluate associations with demographic, clinical and serological characteristics.

Methods: Patients completed the UCLA SCTC GIT 2.0 questionnaire for SSc-GI disease to assess the burden of GI disease across multiple functional and psychological domains. Questionnaire scores were assessed using non-parametric and quantile regression analyses.

Results: Our cohort included 526 patients with SSc, with a typical distribution of disease-associated autoantibodies (ACA, ARA, ATA, PM-Scl, U1RNP, U3RNP). We demonstrated associations between hallmark antibodies and the domain-specific burden of GI disease. In particular, ACA, ARA, and ENA-negative demonstrated increased GI-SSc disease burden, whilst PM-Scl conferred relative protection. In a distributional analysis, associations with autoantibodies were particularly marked in those with the highest burden of GI disease.

Conclusion: There is a significant burden of SSc-GI disease in patients with SSc; reflux and bloating symptoms are most prominent. SSc hallmark antibodies may predict increased risk of SSc-GI disease, in particular ACA and ARA, whilst PM-Scl may be protective.

Scleroderma, autoantibody, gastrointestinal, outcome, patient-reported outcome

Introduction

Gastrointestinal (GI) manifestations are well-recognised as a debilitating complication for many patients with systemic sclerosis (SSc). However, to date, it has been challenging to develop targeted therapies for SSc-GI disease and therefore management is largely symptomatic. In addition to poor understanding of its pathogenesis in general, this can partly be attributed to the clinical heterogeneity of SSc-GI disease: patients present with a wide range of symptoms spanning the length of the GI tract, from gastro-oesophageal reflux to pseudo-obstruction and incontinence.

Pathogenesis of SSc is characterised by microvascular dysfunction, immune cell activation and fibrosis in many tissues. The GI tract is very commonly affected with around 90% of SSc patients manifesting SSc-GI complications (1). For other major complications of SSc, disease-associated antinuclear antibodies predict risk and can function as prognostic markers (2). Antibodies associated with severe GI disease in previous studies include U3RNP (3) and U11/U12RNP (4). In addition, functional muscarinic-3 receptor antibodies and other reactivities have been reported and could contribute to pathogenesis (5,6). Moreover, IVIG has demonstrated improved SSc-GI symptoms which might reflect modulation of functional autoantibodies acting on the gut (7). On the other hand, PM-Scl antibodies have been associated with a lower frequency of certain GI complications such as reflux (8).

In this study, we explored how demographic, clinical and serological characteristics of SSc patients relate to the burden of gastrointestinal symptoms. We used the UCLA SCTC GIT 2.0 (GIT 2.0), a validated SSc-GI disease scoring tool, , to develop predictive models for SSc-GI disease, as have recently been developed for other organ complications such as SSc-related pulmonary fibrosis (2).

Methods

Patient cohort

The Centre for Rheumatology, Royal Free Hospital, is a large tertiary referral centre for SSc. During the study period (2018 - 2020), consecutive SSc patients fulfilling the 2013 ACR/EULAR criteria were recruited. All patients provided written informed consent for this study which was approved by London-Fulham NHS Research Ethics Committee (IRAS ID 279682).

GIT 2.0 questionnaire

The UCLA Scleroderma Clinical Trials Consortium Gastrointestinal 2.0 (GIT 2.0) questionnaire is a validated tool to quantify the burden of SSc-related gastrointestinal symptoms across GI domains, including social functional and emotional wellbeing. For each of 34 items, respondents rate their symptoms over the past seven days on a Likert scale from 0 - 3, with a lower score indicating a better health-related quality of life. There are seven GI domains: reflux (8 items), distension/bloating (4 items; referred to as bloating), faecal soilage (1 item), diarrhoea (2 items), social functioning (6 items), emotional wellbeing (9 items), and constipation (4 items). The total GI score averages 30 items from all domains except constipation (9). Established domain-specific thresholds categorise symptoms into none-to-mild, moderate, and severe.

Autoantibody measurement

Antinuclear antibody reactivity was assessed by Hep2 immunofluorescence pattern with extractable nuclear antigen reactivity determined with counter-immunoelectrophoresis. Anti-RNA-polymerase-III reactivity and anti-fibrillarin reactivity was confirmed by ELISA or immune-precipitation. In cases where multiple autoantibodies were present, the most SSc-

specific antibody was prioritised as in previous studies (2). Cases without an SSc-specific ENA positivity were labelled ENA-negative; ENA-negative cases may have ANA positivity.

Statistical analysis

The Kruskal-Wallis with Dunn's post hoc test, and Wilcoxon Rank Sum test, were used for ordinal variables. We used quantile regression to assess the distribution of GIT 2.0 scores, with dual benefits: assumptions about the distribution of the variable were not required and we could estimate associations between predictor variables and both typical (25^{th} centile, median, 75^{th} centile), and extreme (10^{th} and 90^{th} centile), values across each of the six functional GI domains: total GI score, reflux, bloating, diarrhoea, constipation and soilage(10). Quantile regression was performed using the R package "quantreg" (11). Confidence intervals were calculated using the "xy-pair bootstrap" method with 200 bootstrap replications. In quantile regressions, PM-Scl was chosen as the reference level due to the lowest disease severity and variance. Statistical computations were performed in R version 3.6.3 (2020-02-29).

Results

Patient cohort

Over the study period, 526 patients completed the GIT 2.0 questionnaire. The cohort was 84.4% female, median (IQR) age was 58.0 years, and median (IQR) disease duration was 12.5 (13.3) years. Patients with limited systemic sclerosis (lcSSc) comprised 64.6% of the cohort. Overlap syndromes were seen in 24.5% of patients. 94.2% of respondents were ANA positive; the most commonly observed SSc antibody subtypes were ACA and ATA (31.7% and 22.2% of respondents respectively), followed by ARA (10.5%), U1RNP (5.3%), PM-Scl (4.9%) and U3RNP (3.2%). ENA-negative comprised 24.3%. Other antibodies detected included Ro (8.0%), dsDNA (2.3%), Sm (2.1%), AMA (2.1%), ANCA (1.3%) and La (1.1%).

SSc-GI disease burden

For total GI score, 25.9% reported moderate and 25.7% reported severe SSc-GI burden (Supplementary Table S1). The highest prevalence of severe disease was in the bloating domain (31.0%) followed by the reflux domain (26.5%). Over half of respondents reported at least moderate total GI, reflux, and diarrhoea disease burden. There were no significant differences in any GI domain between limited and diffuse skin subsets (data not shown). Female participants showed significantly higher emotional burden and soilage scores compared to males ($p = 0.009$ and $p = 0.027$, respectively).

Association of SSc-GI disease with antibody subtype

Across domains, including total GI score, bloating, and soilage, antibody subtype was significantly associated with disease burden (total GI score, $p = 0.018$; reflux, $p = 0.05$; bloating, $p = 0.023$; diarrhoea, $p = 0.59$; constipation, $p = 0.68$; soilage, $p < 0.001$). In the total GI score (Fig. 1A), ARA showed increased disease burden compared to PM-Scl ($p = 0.005$), ATA ($p = 0.044$) and U1RNP ($p = 0.02$); likewise, ACA compared to ATA ($p = 0.044$) and

U1RNP ($p = 0.028$); similarly, ENA-negative ($p = 0.02$) and ACA ($p = 0.007$) compared to PM-Scl. For reflux (Fig. 1B), ACA ($p = 0.003$), ATA ($p = 0.027$), ARA ($p = 0.003$), U3RNP ($p = 0.027$) and ENA-negative ($p = 0.004$) showed increased disease burden compared to PM-Scl. For bloating (Fig. 1C), ARA demonstrated increased disease burden compared to PM-Scl ($p = 0.041$). For soilage (Fig. 1F), ACA showed increased disease burden compared to PM-Scl ($p = 0.019$), ENA-negative ($p < 0.001$), ATA ($p < 0.001$) and U1RNP ($p = 0.007$). There were no significant pairwise differences in the diarrhoea (Fig. 1D) or constipation (Fig. 1E) domains.

Quantile regression revealed a distributional association of autoantibodies

We further evaluated the distributional effects of autoantibodies in a quantile regression analysis. For the total GI score (Fig. 2A), antibody subtypes ACA, ARA and ENA-negative demonstrated higher disease burden compared to PM-Scl at quantiles above the median. With ACA, the predicted total GI score at the 90^{th} centile increased by 1.0 points 95% CI [0.14, 1.9] ($p = 0.023$); similarly, with ARA, an increase of 1.1 points 95% CI [0.18, 2.0] ($p = 0.019$) was shown. Notably, potentially harmful antibody subtypes had greater effects on predicted scores at the highest quantiles: in those with the greatest disease burden. For reflux (Fig. 2B), ACA, ARA and ENA-negative demonstrated higher disease burden across multiple quantiles above the 25th centile; ATA and U3RNP showed increased reflux score at the 75^{th} centile. For bloating (Fig. 2C), ACA, ARA and ENA-negative demonstrated higher disease burden, significant at the 75^{th} centile. For diarrhoea (Fig. 2D), ACA showed higher disease burden at the 90^{th} centile but, unlike other domains, there was no discernible trend across other quantiles. Additionally, ATA and ENA-negative demonstrated a trend towards increased diarrhoea score. For constipation (Fig. 2E), ACA, ATA and ENA-negative showed increased disease burden at the 90^{th} centile; ARA showed a significant increase at the median. For soilage (Fig. 2F), ARA and the diffuse skin subtype demonstrated higher disease burden at higher quantiles.

Discussion

This is a large study of over 500 individuals completing a validated SSc-specific GI disease questionnaire. Over half the cohort reported moderate-to-severe disease across the total GI, reflux, and diarrhoea domains. There was also substantial burden of faecal soilage, bloating, constipation, and impact on social functioning and emotional wellbeing. These findings are in agreement with studies of the SSc-GIT 1.0, the previous version of this questionnaire, and other studies of the GIT 2.0 (9,12,13).

Associations between SSc-GI disease and several autoantibodies have been suggested (14); however, previous studies have not demonstrated differences between hallmark SSc antibodies (12). This study is the first to comprehensively explore ANA associations in an unselected cohort of SSc patients with a detailed assessment of GI symptoms. Importantly, we demonstrated that the burden of GI disease across functional GI domains is unevenly distributed amongst hallmark SSc autoantibodies.

ACA was linked to significantly worse GI disease in several domains including total GI, reflux, bloating, constipation, diarrhoea and possibly soilage. Similarly, ARA and ENA-negative were linked to increased SSc-GI disease across multiple domains. Notably, PM-Scl showed the lowest burden of GI disease across GI domains. This fits with previous studies which showed a lower burden of reflux with PM-Scl (8,15). Previously, ACA has been considered protective against the development of internal organ complications of SSc (16). However, recent work has demonstrated the development of late organ complications in the ACA subtype, particularly in those with a diffuse skin type (17). U3RNP has been implicated in severe gastrointestinal disease in small observational studies (18). However, in this cohort, U3RNP was only weakly associated with reflux. However, interpretation is limited by the small size of this antibody group.

The ENA-negative subtype was linked to increased SSc-GI. Despite the negative ENA, there may be undetected antinuclear, or directly pathogenic, antibodies in this group. Anti-myenteric antibodies have been demonstrated in cohorts with SSc-GI disease: antibodies to the muscarinic M3R can block excitatory neurotransmission required for intestinal motility and antibodies to the ganglionic nAChR have also been identified (5,6).

In contrast to other organ-specific complications of SSc, an association between skin subtypes (limited and diffuse) and GI disease has not been reported; likewise, in this study we did not find such an association with GIT 2.0 scores.

Across GI domains, the distribution of GIT 2.0 scores was skewed, with markedly greater variance amongst higher scores (Fig. 1). To explore this distribution, and the relation to antibody subtypes, we used quantile regression. This analysis revealed a population-level impact of antibody subtype, where 'harmful' subtypes markedly increased GIT 2.0 scores at the 75^{th} and 90^{th} centiles, in comparison to modest effects at lower quantiles. This distributional pattern was observed across GI domains and antibody subtypes. One explanation is that certain individuals, by their genetic-environmental background, are more vulnerable to GI disease, which interacts with the 'harmful' antibody subtype. In contrast, a protective background might mediate resilience to the SSc-GI disease mechanism. Thus, antibody subtype is not deterministic of SSc-GI disease burden but, instead, interacts with the genetic-environmental context.

This study was conducted at a single large centre and replication in an independent and multi-centric cohort would strengthen generalisability. In addition, future work might search for additional anti-gut antibodies and to investigate whether such antibodies meet criteria for a pathogenic autoantibody (19).

The GIT 2.0 is a well-validated measure of SSc-GI disease, however, it is limited by the assessment of symptom frequency rather than severity, and that treatments, GI investigations, laboratory tests, and clinical parameters (such as weight loss) are not included. However, this second version of the GIT 2.0 includes faecal soilage, an important outcome for patients, enhancing its face validity. Additionally, studies comparing the GIT 2.0 with objective GI investigations have demonstrated concurrent validity: oesophageal investigations including manometry and scintigraphy correlate specifically with the reflux scale (20).

In conclusion, GI disease is prevalent in SSc and has a significant impact on social functioning and emotional wellbeing. Antibody subtypes confer differing risk of GI disease burden, most prominent in those with the most severe GI disease. The mechanisms relating antibody subtype to SSc-GI phenotype remain unclear. Underlying disease mechanisms including microvascular damage and tissue fibrosis, that may differ between ANA subtype, might drive SSc-GI disease or the hallmark SSc-associated ANA reactivities may be linked to pathogenic anti-gut antibodies. If so, this may have therapeutic implications for use of antibody or B-cell-directed treatment.

Key messages

- A patient-reported outcome reliably captures SSc-GI burden, which is moderate or severe in over half.
- Female patients showed significantly higher emotional burden and faecal soilage scores compared to males.
- SSc-autoantibodies ACA and ARA associated with more severe SSc-GI disease, and PM-Scl had lower severity.

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Table and Figure Legends

Figure 1 A-F. GIT 2.0 domain scores by autoantibody subtype.

Individuals with SSc (n = 526) completed the GIT 2.0 questionnaire of GI disease burden across functional GI domains. Boxplots demonstrate (A) total GI, (B) reflux, (C) bloating, (D) diarrhoea, (E) constipation and (F) soilage domain scores by autoantibody subtype. Across GI domains, ACA and ARA were associated with significantly increased disease burden, in contrast to PM-Scl, with the lowest burden of SSc-GI disease. Statistical analysis: groupwise comparisons by Kruskal-Wallis test; pairwise comparisons by Dunn's post hoc test. Significant pairwise differences ($p < 0.05$) from the comparator group (marked with an arrowhead) are indicated with an asterisk.

Figure 2 A-F. Quantile regression analysis of GIT 2.0 domain scores by autoantibody and skin subtypes.

Quantile regression coefficients for subtypes (autoantibody and skin) across GIT 2.0 domains: (A) total GI, (B) reflux, (C) bloating, (D) diarrhoea, (E) constipation and (F) soilage. Quantile regression revealed that, compared to PM-Scl (reference level), the ACA, ARA and ENA-negative subtypes were associated with increased scores across GI domains, and ATA showed a trend towards increased disease burden. Regression coefficients were markedly larger at the highest quantiles (75^{th} and 90^{th} centiles), revealing a distributional association with subtypes. Statistical analysis: quantile regression coefficients were estimated at five quantiles from 0.1 to 0.9. Error bars indicate 95% confidence intervals; those not crossing zero are statistically significant, indicated with an asterisk.

Supplementary Table S1. Burden of GI disease across the GIT 2.0 domains.

Participants (n = 526) completed the 34-item GIT 2.0 questionnaire of SSc-GI disease burden. Symptoms, across seven GI domains, were rated from 0 - 3, with a lower score indicating a

better health-related quality of life. The total GI score averages 30 items from all domains, excluding constipation. Established domain-specific thresholds categorise symptoms into mild, moderate, and severe.

Tables and Figures

Figure 1 A-F.

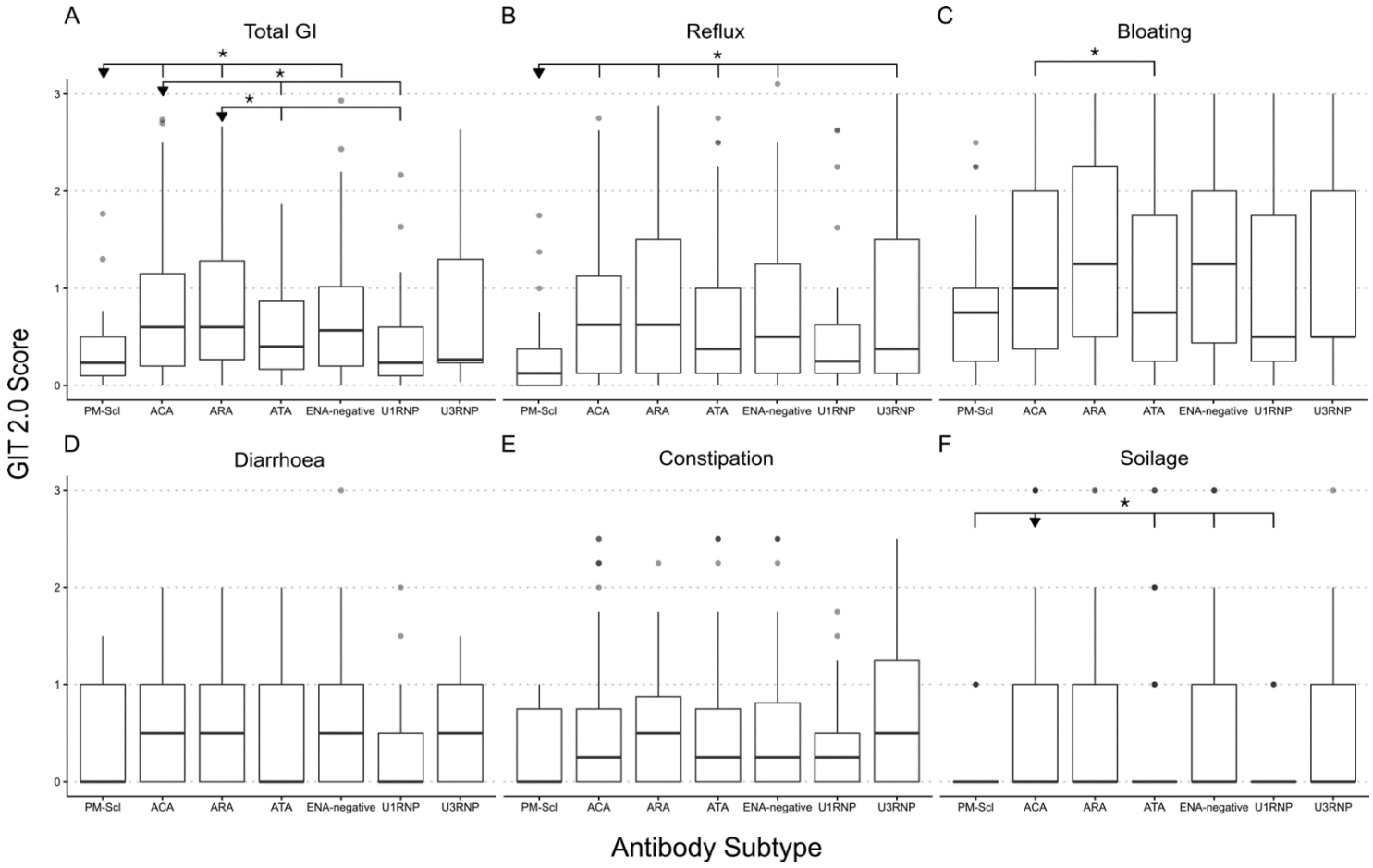
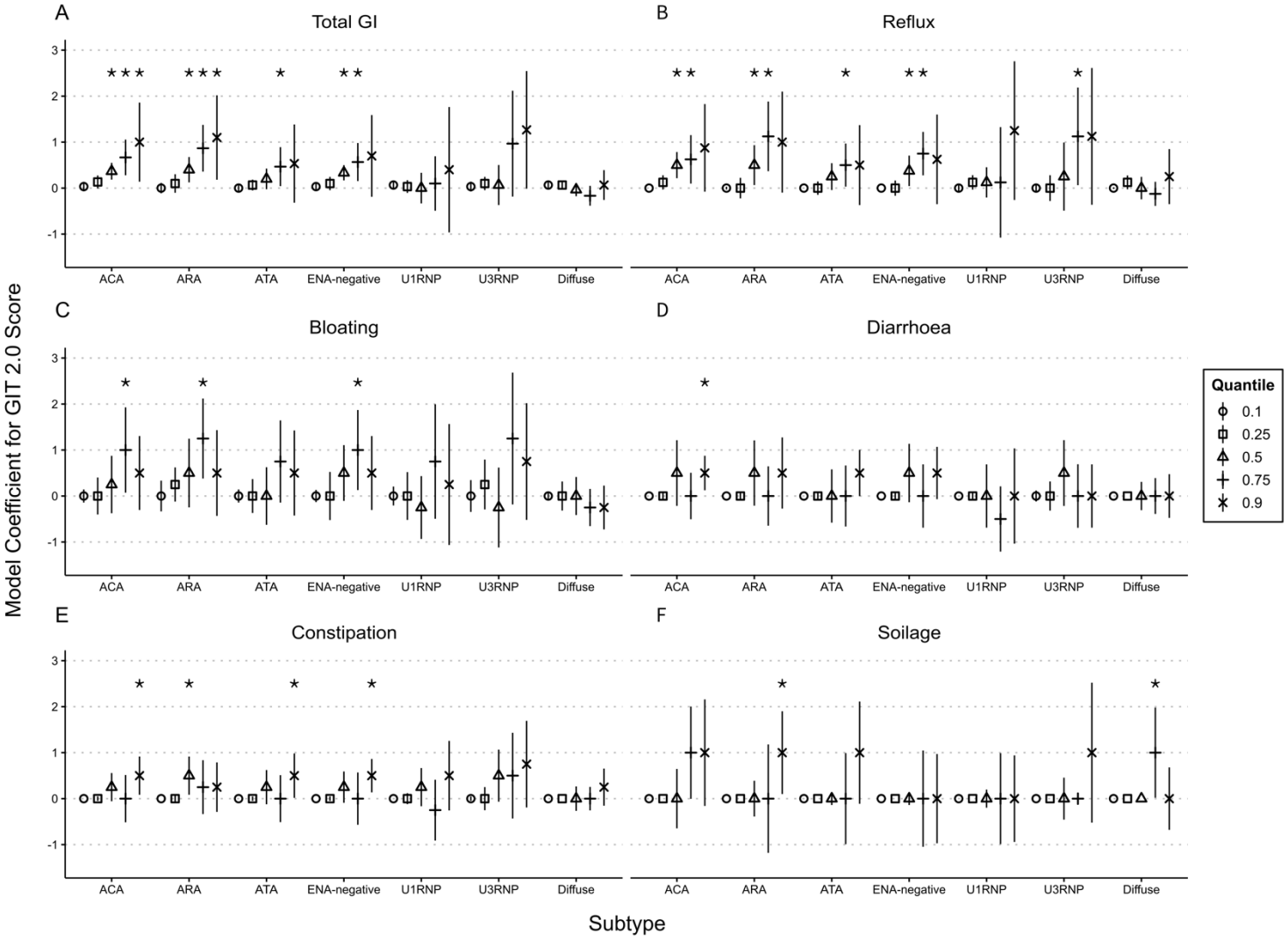


Figure 2 A-F.


A
Total GI
B
Reflux
C
Bloating
D
Diarrhoea
E
Constipation
F
Soilage
Model Coefficient for GIT 2.0 Score
Subtype
ACA
ARA
ATA
ENA-negative
U1RNP
U3RNP
Diffuse
Quantile
0.1
0.25
0.5
0.75
0.9

Supplementary Material

Supplementary Table S1.

GIT 2.0 domain	Mean (SD)	Median (IQR)	None-to-mild; n (%)	Moderate; n (%)	Severe; n (%)
Total GI	0.67 (0.61)	0.50 (0.87)	254 (48.4)	136 (25.9)	135 (25.7)
Reflux	0.73 (0.73)	0.50 (1.0)	247 (47.0)	139 (26.5)	139 (26.5)
Bloating	1.2 (0.95)	1.0 (1.8)	281 (53.4)	82 (15.6)	163 (31.0)
Diarrhoea	0.50 (0.61)	0.50 (1.0)	260 (49.5)	193 (36.8)	72 (13.7)
Constipation	0.50 (0.60)	0.25 (0.75)	282 (53.7)	168 (32.0)	75 (14.3)
Soilage	0.52 (0.84)	0.0 (1.0)	452 (85.9)	48 (9.1)	26 (4.9)
Emotional	0.62 (0.82)	0.22 (0.89)	319 (60.8)	86 (16.4)	120 (22.9)
Social	0.44 (0.61)	0.17 (0.67)	324 (61.6)	129 (24.5)	73 (13.9)