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Genetic risk factors for perception of symptoms in GERD: an observational cohort study

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Summary

Background: Genetic polymorphisms in G-protein beta-3 subunit (GN β 3) and beta-2 adrenergic receptor (ADRB2) are associated with pain and gut hypersensitivity, which can overlap with gastroesophageal reflux disease (GERD).

Aim: To evaluate relationships between single nucleotide polymorphisms (SNPs) within GN β 3 and ADRB2 systems, and reflux symptom burden, GERD phenotypes from ambulatory reflux monitoring, and quality of life.

Methods: Symptomatic adults undergoing ambulatory reflux testing were recruited and phenotyped based on acid burden and symptom reflux association; major oesophageal motor disorders and prior foregut surgery were exclusions. A comparison asymptomatic control cohort was also identified. Subjects and controls completed questionnaires assessing symptom burden on visual analog scales, short-form health survey-36 (SF-36), and Beck Anxiety and Depression Inventories (BAI and BDI). Genotyping was performed from saliva samples; 6 SNPs selected from each of the two genes of interest were compared.

Results: Saliva from 151 study subjects (55.3 ± 1.2 years, 63.6% F) and 60 control subjects (50.9 ± 2.2 years, 66.7%) had sufficient genetic material for genotyping. Study subjects had higher symptom burden, worse total and physical health, and higher anxiety scores compared to controls ($P = .002$). Tested SNPs within ADRB2 were similar between study subjects and controls ($P > .09$). Study subjects with recessive alleles in 3 GN β 3 SNPs (Rs2301339, Rs5443, and Rs5446) had worse symptom severity ($P = .011$), worse mental health ($P = .03$), and higher depression scores ($P = .005$) despite no associations with GERD phenotypes or reflux metrics.

Conclusions: Genetic variation within GN β 3 predicts oesophageal symptom burden and affect, but not oesophageal acid burden or symptom association with reflux episodes.

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SUPPORTING INFORMATION

Additional Supporting Information will be found online in the supporting information tab for this article.

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1 | INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common outpatient gastrointestinal diagnosis, with estimates of prevalence of 10%-30% in the Western world.^{1,2} At its core, GERD is suspected on the basis of troublesome oesophageal symptoms; mucosal injury (oesophagitis, intestinal metaplasia) is not consistently encountered, even in treatment-naïve patients.³ Despite this symptom-based paradigm, many GERD patients do not consistently report symptom events in close temporal relationship to reflux episodes identified on ambulatory reflux monitoring, and associating symptom events with reflux episodes is considered the weakest link in reflux monitoring.^{4,5} This phenomenon is at odds with the prime indication for ambulatory reflux monitoring: persistent oesophageal symptoms despite seemingly adequate reflux management. Symptom-reflux association, and indeed symptom severity, does not necessarily correlate with acid exposure time (AET);⁶ on the contrary, positive symptom-reflux association in the setting of physiologic AET may be associated with lower oesophageal perceptive thresholds and less favourable response to antireflux therapy.^{7,8}

Recent data suggest the presence of a genetic component to gut visceral perception. Genetic polymorphisms in the secondary messenger gene G-protein beta-3 subunit (GN β 3)⁹ and beta-2 adrenergic receptor system (ADRB2)¹⁰ are reported to predict enhanced perception in functional disorders, including those involving the foregut and overlapping with GERD.⁹ However, it remains unclear if single nucleotide polymorphisms (SNPs) within the GN β 3 and/or ADRB2 systems influence the severity of oesophageal symptom perception and segregate GERD phenotypes based on objective reflux metrics.¹¹ Furthermore, to our knowledge, the relationships between these SNPs and affective and health-related quality of life metrics have not previously been systematically investigated among a cohort of patients undergoing ambulatory reflux monitoring for suspected GERD symptoms.

In this exploratory study, we hypothesised that certain genetic polymorphisms within the GN β 3 and ADRB2 systems predict increased perceptive symptom burden in patients with persisting oesophageal symptoms referred for ambulatory reflux monitoring. Our primary aim was to assess the relationship between the relative frequencies of selected SNPs in the ADRB2 and GN β 3 systems with GERD phenotypes, oesophageal symptom severity, and health-related quality of life (HRQOL) using validated questionnaires, and to compare these results to similar data from controls without oesophageal symptoms.

2 | METHODS

2.1 | Subjects and recruitment

Symptomatic adult patients referred for ambulatory reflux monitoring (pH- or pH-impedance testing) who provided a saliva sample for genetic interrogation were eligible for inclusion in this exploratory pilot study. Patients were recruited from two main settings: patients referred for oesophageal physiologic testing in the context of persisting reflux symptoms (both typical and atypical) despite acid suppression, and symptomatic patients being evaluated to document pathologic oesophageal reflux burden prior to antireflux surgery or invasive reflux therapy. Exclusion criteria consisted of major oesophageal motor disorders (eg, achalasia, absent contractility, diffuse oesophageal spasm, hypercontractile

oesophagus),¹² prior foregut surgery, and incomplete or artifactual reflux studies. A control cohort was identified from our institutional databank and tissue repository consisting of individuals who reported no oesophageal symptoms on validated questionnaires,¹⁰ and no proton pump inhibitor (PPI) usage reported on questionnaire or recorded on chart review; these patients were collected as normal controls for previous genotype-phenotype studies involving functional gastrointestinal disorders performed at our center.¹⁰ To our knowledge, no subjects were first-degree relatives of the enrolled study or control cohorts. All subjects and controls provided written informed consent prior to participation. This study protocol was approved by the Human Research Protection Office (Institutional Review Board, IRB) at Washington University in St. Louis.

2.2 | Symptom burden

Subjects and controls completed several questionnaires categorising oesophageal and foregut symptoms. Self-reported symptom burden (symptom severity and bother) was evaluated within the previous 2 weeks on 10-cm visual analog scales (VAS), using previously described methodology.¹⁰ Symptom frequency (number of symptomatic days) within the proceeding 2 weeks was quantified (0-14 days). The Beck anxiety and Beck depression inventories (BAI, BDI) were also completed, with scores ranging from 0 (minimal) to 63 (the most severe).¹³ The short-form health survey 36 (SF-36), a validated assessment of physical and mental health status, was utilised to assess health-related quality of life (HRQoL), where a score of 0 indicates maximal disability and 100 indicates no disability.¹⁴

2.3 | Oesophageal studies

Ambulatory reflux monitoring was performed using either catheter-based (pH or pH-impedance) or wireless pH techniques, with consistent placement of the pH sensor 5 cm proximal to the manometrically identified lower oesophageal sphincter (LES) for catheter-based studies or 6 cm proximal to the squamocolumnar junction for wireless pH monitoring. Ambulatory reflux testing at our center is “open-access,” and referring physicians decide whether testing should be performed on or off antisecretory therapy. When testing is performed off therapy, patients are instructed to stop PPIs 7 days prior to the study, and histamine-2 receptor antagonists, prokinetic medications and antacids 3 days prior to the study. Antisecretory medication status at the time of the testing was recorded.

Raw pH and pH-impedance studies were interrogated using manufacturer-specific software, and mealtimes were excluded from analysis. The primary metric extracted was the AET, the percentage of time the pH was below 4 at the distal oesophageal pH sensor. AET <4.0% was designated normal per our institutional threshold^{15,16}; abnormal AET was further segregated into the following values: 4%-6% (borderline elevation) and >6% (pathologic elevation).¹⁷ Symptom association probability (SAP) was calculated using the Ghillebert probability estimate (GPE),¹⁸ and designated as positive if the likelihood of a chance association between the symptom and reflux episodes was <5%, corresponding to $P < .05$. Our group has previously shown that the GPE correlates very well with SAP calculated using the Weusten method, with sensitivity 0.95 and specificity 0.91.^{19,20} Based on AET and SAP, evidence for GERD was categorised into the following GERD phenotypes:¹¹ (1) strong (abnormal AET and positive SAP), (2) good (abnormal AET but negative SAP), (3) reflux

hypersensitivity (RH; normal AET but positive SAP), and (4) no evidence (negative AET and SAP).

2.4 | DNA sample collection and genotyping

DNA was extracted from sputum samples with Qiagen Gentra Puregene reagents (Qiagen Inc., Valencia, CA), then frozen and banked by the Biobank Core of the Washington University Digestive Diseases Research Center. ADRB2 and GN β 3 were selected for investigation because previous studies suggested the potential importance of these gene systems in visceral perception, particularly in the foregut and with GERD symptoms.^{9,10} Six SNPs from each of the ADRB2 and GN β 3 systems (rs1042713, rs1042714, rs1042717, rs1042718, rs1042719, rs1801704; rs5442, rs5443, rs5445, rs5446, rs2301338, rs2301339) were analysed. These particular SNPs were selected because of their focus in previous work and/or their location and distribution of alleles on the International Hapmap Project (<https://hapmap.ncbi.nlm.nih.gov/>).²¹ Genotyping was performed with the Biomark HD system (Fluidigm, South San Francisco, CA) in a single-batch run containing all study samples (both cases and controls). The laboratory staff was blinded to study hypotheses and study groups.

2.5 | Data analysis

Data are reported as the mean $\pm$ standard error of the mean, and 95% confidence intervals (CI), unless otherwise noted. Categorical data were compared using the chi-square test; continuous data were compared using ANOVA or the two-tailed Student's *t* test, as appropriate. A multivariate logistic regression model was created to predict the presence of any of the alleles of interest. In all cases, $P < .05$ was required for statistical significance.

Based on prior work at our institution, where 68.3% of patients with functional gastrointestinal disorders and 55.4% of control subjects were rs1042714 G allele carriers,¹⁰ we proposed a sample size of 188 subjects for each group (study cohort and control cohort), which would have a power of 80.1% to yield a statistically significant result (using a criterion for significance of $\alpha = 0.050$). Although we appropriately enrolled 193 study subjects, our strict criteria for our control cohort (absence of esophageal symptoms on questionnaires or PPI use) resulted in the enrollment of only 98 control subjects from our existing institutional databank and tissue repository.

Hardy-Weinberg equilibrium was assessed for each examined SNP.²² While all ADRB2 SNPs were in Hardy-Weinberg equilibrium for the entire cohort, controls, and study patients ($P > .1$), several GN β 3 SNPs were in Hardy-Weinberg disequilibrium for the entire cohort (Rs2301338, Rs2301339, Rs5443, Rs5445), controls (Rs5442, Rs5443, Rs5445, Rs5446), and study patients (Rs 2301338, Rs5445) ($P < .04$; Tables S1-S3). However, within the study cohort with GERD symptoms, the 3 SNPs that we focus on in this manuscript (Rs2301339, Rs5443, and Rs5446) were in Hardy-Weinberg equilibrium ($P > .25$). Small proportions of recessive alleles (particularly Rs2301338, Rs5442, and Rs5445), population stratification, and sampling error may have contributed to the disequilibrium.²³ While acknowledging the presence of disequilibrium and these potential etiologies, we felt the exploratory nature of

this study justified proceeding with SNP analysis.^{22,24} Statistical analyses were performed using IBM SPSS Statistics V.23 (Armonk, NY).

3 | RESULTS

3.1 | Subjects

One hundred and ninety three study patients were enrolled, of which 42 had inadequate genetic material concentrations to undergo SNP analysis. Of 98 potential controls, 38 had insufficient genetic material concentrations for analysis. Therefore, our study cohort consisted of 151 study subjects (55.3 ± 1.2 years, 63.6% females) and 60 controls (50.9 ± 2.2 years, 66.7% females); study and control groups shared similar demographics ($P > .08$ for each comparison) (Figure 1). Within the study cohort, 132 (87.4%) were tested off PPI therapy. Dominant typical reflux symptoms (heartburn or regurgitation) were reported by 65.6%; the remainder had atypical symptoms. When stratified into GERD phenotypes, there were 50 subjects with strong evidence of reflux, 34 with good evidence, 16 with RH, and 51 with no evidence of reflux (Figure 1). Demographics (age, gender, and race) were similar between the four phenotypes ($P > .25$, Table 1).

3.2 | Symptom burden and HRQoL

Questionnaire data were complete in 123 study subjects and all 60 controls. Compared to controls, study subjects had significantly higher symptom burden metrics ($P = .002$), lower total and physical SF-36 scores ($P = .001$), higher BAI scores ($P < .001$), but similar mental SF-36 scores ($P = .87$), and BDI scores ($P = .08$, Figure 2). Higher symptom burden metrics were reported with typical reflux symptoms compared to atypical symptoms (symptom bother: ± 0.3 vs 4.4 ± 0.5 , $P = .007$; symptom severity: 6.3 ± 0.3 vs ± 0.5 , $P = .049$); in contrast, SF-36 scores ($P > .13$ for all comparisons), BAI ($P = .41$), and BDI ($P = .36$) were similar. Symptom burden, SF-36 scores, BAI, and BDI were similar across GERD phenotypes ($P = .23$, Table 1). Furthermore, these parameters remained similar when compared among patients separated into 3 AET categories¹⁷: AET <4%, AET of 4%-6%, and AET > 6% ($P = .12$ for all comparisons).

3.3 | Distribution of ADRB2 and GNβ3 polymorphisms

Of the genotyped cohorts, 5 (2.4%, 3 study subjects, 2 control) could not be accurately genotyped for a majority of the polymorphisms (3 with 12 “no calls” and 2 with 11 “no calls” among the 12 SNPs), and were excluded from further analysis. Allelic distributions in three SNPs within GNβ3 were significantly different between the study and control cohorts for Rs2301339 ($P = .040$), Rs5443 ($P = .011$), and Rs5446 ($P = .016$) (Table 2), while distributions within the remaining SNPs studied within GNβ3 and all tested ADRB2 SNPs were similar ($P > .09$ for all comparisons). Significant overlaps were noted in allelic distributions between these three SNPs of interest: all 76 subjects who were Rs2301339 recessive (A) allele carriers were also Rs5443 recessive (T) allele carriers; 97.0% and 98.5% of the 67 Rs5446 recessive (T) allele carriers were also Rs2301339 A allele carriers and Rs5443 T allele carriers, respectively. For Rs2301339 and Rs5443, significantly higher proportions of each major homozygote (GG, CC, and CC, respectively) were Caucasian ($P < .001$ for each comparison).

Within the three SNPs of interest (Rs2301339, Rs5443, Rs5446), recessive allele carriers (A, T, and T, respectively) had worse symptom severity and BDI ($P = .02$), and trended towards worse mental SF-36 scores ($P < .07$) and symptom bother ($P < .1$) compared to their corresponding dominant alleles (Figure 3). Demographics, clinical presentation ambulatory reflux monitoring metrics, total and physical SF-36, and BAI scores were similar between recessive and dominant alleles ($P = .14$ for all comparisons). Study subjects who carried all three recessive alleles had higher symptom severity ($P = .01$), lower mental SF-36 scores ($P = .03$), and higher BDI ($P = .005$), but similar total SF-36 scores ($P = .35$), physical SF-36 scores ($P = .8$), and BAI ($P = .24$) compared to those with all three dominant alleles. In contrast, the presence of any combination of these three recessive alleles was not associated with differences in GI symptom severity among the control group without oesophageal symptoms ($P = .16$).

Allelic distributions of the 12 tested polymorphisms were similar across GERD phenotypes, and no associations were identified with any of Rs2301339, Rs5443, and Rs5446 and GERD phenotypes ($P = .7$). Allelic distributions between patients with and without positive symptom-reflux association ($P > .2$), and between patients with dominant perceptive (heartburn, chest pain) and non-perceptive (regurgitation) symptoms ($P > .07$) were also similar.

3.4 | Subgroup analysis: study subjects tested Off PPI

Of the 151 study subjects, 132 (87.4%; 55.3 ± 1.3 years, 62.1% female, 68.9% with dominant typical presenting symptoms) who underwent reflux monitoring off PPI therapy were further analysed separately. None of the allelic frequencies of the 12 analysed SNPs demonstrated any significant association with the distribution of GERD phenotypes on χ^2 testing ($P > .18$ for all comparisons). However, allelic distributions among the three SNPs of interest within GN β 3 predicted symptom burden along similar lines as seen for the entire cohort. The presence of the recessive A allele in Rs2301339 again predicted worse symptom severity ($P = .023$), symptom bother ($P = .034$), and BDI ($P = .018$), while trending towards lower mental SF-36 ($P = .094$). The presence of the recessive T allele in Rs5443 predicted worse symptom severity ($P = .027$), symptom bother ($P = .047$) and BDI ($P = .037$), but not worse mental SF-36 ($P = .14$). The presence of the recessive T allele in Rs5446 predicted symptom severity ($P = .043$), and trended towards worse symptom bother ($P = .052$), mental SF-36 ($P = .09$) and BDI ($P = .086$).

3.5 | Multivariate predictors of recessive alleles

Logistic regression models were created to predict the presence of any of the three recessive alleles of interest among the study subjects, with AET, positive SAP, BAI, BDI, symptom severity and physical and mental SF-36 scores included as independent variables. Among these variables, only worse BDI scores ($P = .023$) and increased symptom severity ($P = .043$) were significant independent predictors of the recessive alleles within GN β 3; the other variables were not ($P > .11$) (Figure 4). Among only the subjects tested off PPI, BDI scores ($P = .081$) and symptom severity ($P = .067$) trended towards predicting recessive alleles (Figure 4).

4 | DISCUSSION

In this report, we demonstrate that three SNPs from GN β 3, a G-protein coupled receptor whose activation results in increased signal transduction within the brain-gut axis, differ in allelic distributions between patients undergoing ambulatory reflux testing and a cohort of controls without oesophageal symptoms. Moreover, despite similar levels of oesophageal acid exposure and symptom-reflux association compared to the dominant homozygotes, recessive allele carriers of these three SNPs report worse symptom severity, mental health-related quality of life, and Beck Depression Inventory scores among symptomatic reflux patients. Furthermore, worse symptom severity and BDI scores were independently predictive of the presence of these recessive alleles on multivariate regression. Our findings in this exploratory study suggest the presence of specific genotypes predicting increased oesophageal symptom perception and potential hypervigilance, but not increased reflux burden.

Existing literature indicates a lack of direct correlation between oesophageal symptom severity and reflux burden, suggesting variability in oesophageal symptom perception between GERD patients,^{6,19} a concept which our findings support. Increased perception of reflux episodes is noted in some patients with physiologic reflux burden, now termed reflux hypersensitivity and designated a Rome IV functional oesophageal disorder.²⁵ These patients demonstrate lower perceptive thresholds for discomfort with oesophageal balloon distension compared to healthy controls, patients with elevated AET, or those with Barrett's oesophagus.⁷ Even when increased perception is to acid reflux episodes, symptomatic outcome from antireflux therapy is suboptimal;⁸ improvement is similarly suboptimal in functional heartburn.²⁶ In both of these symptomatic functional oesophageal disorders, treatment with neuromodulators may have value.²⁵ Reflux hypersensitivity, and indeed functional heartburn, may overlap with true GERD, indicating that certain patients within the GERD spectrum may be predisposed to increased oesophageal perception.²⁵ This finding lends further support to the existence of a perceptive phenotype, with or without abnormal reflux burden, where oesophageal symptoms persist despite adequate acid suppression, but may respond to approaches directed at functional gastrointestinal disorders.

There is limited evidence in the literature for genetic predisposition to symptomatic oesophageal disorders in the realm of reflux disease. A postal questionnaire study showed familial clustering of reflux symptoms in relatives of patients with reflux symptoms and abnormal AET.²⁷ Likewise, within-pair association for reflux symptoms is stronger among monozygotic than dizygotic twin pairs.^{28,29} Recent genome-wide association studies (GWAS) have afforded insight into the shared genetic background of GERD, Barrett's oesophagus, and oesophageal adenocarcinoma.³⁰ Furthermore, a high-quality GWAS meta-analysis of Northern European cohorts (2247 GERD cases and 4503 asymptomatic controls, assigned based on questionnaire data) identified 30 independent GERD-suggestive risk loci with involvement in plausible relevant biological processes (such as ion channel regulation and cell adhesion), though none of these loci cleared the bar for genome-wide significance.³¹ We speculate that the genetic predisposition observed in our study may reflect perception of reflux symptoms, rather than oesophageal GERD pathophysiology.

Further evidence supporting a symptom-based predisposition is provided by de Vries et al, who report that rs5443, a SNP within the $GN\beta 3$ gene with prior association with hypertension, obesity, and functional dyspepsia (FD), may be associated with symptomatic GERD.⁹ Specifically, this association appeared stronger among patients with heightened perception of physiologic amounts of reflux, supporting the concept that the Rs5443 SNP may facilitate augmented perception of visceral pain in the oesophagus. This SNP is involved with post-synaptic activation of G proteins in gut signaling - TT alleles are associated with increased signal transduction, and CC with reduced signal transduction.^{9,32} Our findings that T allele carriers had worse symptom burden is in keeping with these concepts regarding the Rs5443 SNP. Thus, a partially-genetically determined increased perception of reflux events could therefore result in higher symptomatic burden in GERD.³² Although the allelic distributions of Rs5443 differed between de Vries et al and ours,⁹ our findings corroborate numeric increases in Rs5443 heterozygotes among patients with reflux symptoms when compared to controls.

Therefore, genetic factors may contribute to inter-individual variability in oesophageal perception leading to symptomatic GERD presentations, and genetic polymorphisms may be potentially identifying a state of oesophageal hypervigilance. We demonstrate in this study that certain recessive alleles within the $GN\beta 3$ system are associated not only with increased symptom burden, but also with higher scores on BDI, emphasising the role of the brain-gut axis in noxious GI symptom experiences. Associations between depression and functional GI disorders (particularly IBS), GI symptom burden, and HRQoL are well known.³³ $GN\beta 3$ SNPs have been implicated in mood disorders (albeit inconsistently across different populations); for example, allele frequencies in rs5443 among the Chinese Han population increase susceptibility to and predict antidepressant response in major depression.^{34,35} Furthermore, the recessive T allele at rs5443 may predict the efficacy of nortriptyline, a tricyclic antidepressant, in improving neurovegetative symptoms such as sleep and appetite among patients with depression.³⁶ Within ADRB2, previous data suggest that minor alleles at rs1042714 predict functional gastrointestinal disorders, impacting bowel symptom severity and HRQoL;¹⁰ however, we were unable to demonstrate associations between the studied ADRB2 SNPs and oesophageal symptom burden in this study. These data raise intriguing questions pertaining to the role of genetic predisposition in hypervigilance and symptom perception, not limited to the oesophagus but across the spectrum; these concepts could potentially impact the management of symptomatic gastrointestinal disorders.

Our study has limitations that could have impacted our findings and conclusions. First, in this exploratory study, we utilised a convenience cohort of patients undergoing reflux monitoring, rather than a consecutive cohort, which could have influenced our findings. Furthermore, our sample sizes were limited by our time and cost constraints, lessening the strength of our findings. As subjects were enrolled only from our tertiary referral academic center, our study cohort was more representative of the clinical population referred for evaluation, rather than the overall GERD population, and our findings may have limited generalisability in this respect. Some patients, especially those with previously banked specimens, had tissue samples with insufficient DNA concentrations for genotyping and were excluded. We do not have information on mucosal integrity of the oesophageal mucosa, macroscopic or microscopic, at the time of reflux monitoring - we recognise that

there could be a relationship between mucosal integrity and genotype data that our study was not designed to evaluate. Finally, some of the studied SNPs showed disequilibrium from Hardy-Weinberg (as did GN β 3 Rs5443 in the report by de Vries et al);⁹ this could also have impacted our findings. Nevertheless, our findings suggest a genetic basis for the severity of oesophageal symptoms rather than reflux burden, a finding that harmonises with previous data in the literature, and provides a basis for future hypothesis-driven genetic studies within this field.

In summary, three SNPs from GN β 3, a G-protein coupled receptor involved in signal transduction in the brain-gut axis, demonstrate varying allelic distributions between patients with reflux symptoms relative to controls without oesophageal symptoms. Moreover, among patients with reflux symptoms, recessive allele carriers of these three SNPs have worse symptom severity, mental HRQoL, and depression metrics compared to dominant homozygotes, suggesting a hypervigilant population subset. Our findings highlight the potential for genotyping of oesophageal symptoms within the realm of GERD, and should help guide future inquiry within this area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Study flow diagram describing study subjects and control. Study subjects were phenotyped based on oesophageal acid exposure time (AET, abnormal if $\geq 4\%$) and positive symptom association probability (SAP) into strong evidence (abnormal AET, positive SAP), good evidence (abnormal AET, negative SAP), reflux hypersensitivity (normal AET, positive SAP), and equivocal or no GERD evidence (both metrics negative)

FIGURE 2.

Comparison of symptom burden, health-related quality of life (HRQoL) and affective state between study subjects and controls. Study subjects had worse symptom severity and bother compared to controls, as well as total and physical (but not mental) HRQoL according to SF-36 questionnaires. Study subjects had worse anxiety scores per the Beck anxiety inventory (BAI) but similar depression scores per the Beck depression inventory (BDI)

FIGURE 3.

Relationships between acid burden, symptom metrics, affect, and genotype. Subjects with any recessive alleles for three SNPs within the GNB3 gene had significantly higher GI symptom burden as well as higher scores on the beck depression inventory (BDI) compared to subjects with dominant alleles for the same SNPs ($P < .03$). Furthermore, they either had or trended towards worse mental Short Form 36 (SF-36) scores ($P = .066$, $P = .055$, $P = .031$); physical SF-36 scores and acid exposure time (AET) were similar

FIGURE 4.

Independent predictors of recessive alleles in GN β 3 SNPs of interest. Multivariate logistic regression models were created with any of the three recessive alleles (Rs2301339, Rs5443, Rs5446) as the outcome variables, and included acid exposure time (AET), symptom association probability (SAP), Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), symptom burden, and physical and mental SF-36 scores as independent variables. For all subjects, worse BDI and symptom burden scores significantly predicted the presence of the recessive variables. For subjects tested off PPI therapy, BDI and symptom burden trended towards significance in predicting the presence of the recessive alleles

TABLE 1

Characteristics of GERD phenotypes based on ambulatory reflux monitoring

	Strong reflux evidence n = 50	Good reflux evidence n = 34	Reflux hypersensitivity n = 16	Equivocal or No reflux evidence n = 51	P values ^a
Mean age (y)	54.7 ± 2.0	54.6 ± 2.4	50.1 ± 4.4	58.0 ± 1.9	.25
Sex (F)	32 (64.0%)	19 (55.9%)	12 (75.0%)	33 (64.7%)	.61
Race (Caucasian)	46 (92.0%)	30 (88.2%)	16 (100%)	43 (84.3%)	.29
Symptom severity VAS	6.3 ± 0.5	5.2 ± 0.7	5.8 ± 1.0	4.9 ± 0.5	.23
Symptom bother VAS	6.4 ± 0.5	5.4 ± 0.6	6.3 ± 0.9	5.6 ± 0.5	.48
Total SF-36	54.8 ± 3.4	49.8 ± 3.6	61.4 ± 6.9	55.4 ± 3.8	.52
Physical SF-36	47.5 ± 3.6	49.8 ± 3.9	53.7 ± 7.5	48.8 ± 3.9	.46
Mental SF-36	76.0 ± 2.6	67.7 ± 4.5	72.3 ± 6.7	70.0 ± 3.0	.37
BAI	12.2 ± 1.3	12.7 ± 1.6	13.4 ± 2.9	11.4 ± 1.5	.90
BDI	8.5 ± 0.8	10.5 ± 1.6	8.2 ± 2.3	9.3 ± 1.3	.69
AET (%)	10.1 ± 0.8	14.4 ± 2.6	1.8 ± 0.3	0.8 ± 0.1	<.001

By definition, all subjects with strong GERD evidence and reflux hypersensitivity have a positive symptom association probability (SAP), all other groups have a negative SAP.

VAS, visual analog scale; SF-36, short form-36; BAI, Beck anxiety inventory; BDI, Beck depression inventory; AET, acid exposure time.

^aANOVA for continuous variables, chi-square for categorical variables.

TABLE 2

Selected genotype distributions for study subjects and controls

	Study subjects n = 151	Control subjects n = 60	P values ^a
Rs2301339			.040
AA	18 (12.2%)	16 (27.6%)	
GA	58 (39.2%)	21 (36.2%)	
GG	71 (48.0%)	20 (34.5%)	
Rs5443			.011
CC	71 (48.0%)	20 (34.5%)	
TC	59 (39.9%)	21 (36.2%)	
TT	18 (12.2%)	17 (29.3%)	
Rs5446			.016
CC	77 (52.0%)	25 (43.1%)	
TC	57 (38.5%)	19 (32.8%)	
TT	10 (6.8%)	13 (22.4%)	

^aChi-square test.