Title of the study: Functional Dyspepsia Treatment Trial
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Abstract

Functional dyspepsia is a common, chronic and costly disorder that substantially impairs quality of life for many Americans. The pathogenesis is unclear but abnormalities of gastric motor and sensory function have been identified; it is uncertain if the recognized physiological abnormalities influence treatment response. Furthermore, twin studies suggest there is a genetic component, and we have novel pilot data suggesting that a heterotrimeric G protein polymorphism is associated with functional dyspepsia and may predict response to therapy. The currently available therapies for functional dyspepsia are very unsatisfactory.

We propose to investigate whether antidepressant medications are efficacious in functional dyspepsia. The use of antidepressants to treat functional dyspepsia is based on three propositions. First, antidepressants could reduce the severity of co-morbid psychological symptoms, especially anxiety and depression. Second, antidepressants have central analgesic actions. Thirdly, antidepressants have been shown to have local pharmacological actions on the gut, and may specifically alter gastric emptying and fundic relaxation based on preliminary data, but the relevance of such pertubations to treatment outcome is not established.

Research Plan

Hypothesis and Specific Aims

In functional dyspepsia:
1. The tricyclic antidepressant amitriptyline in low dose (50mg), and the selective serotonin reuptake inhibitor (SSRI) escitalopram in standard dose (10mg), will be superior to placebo in terms of global symptom relief as judged by the patient, adjusting for psychological and psychiatric co-morbidity. Moreover, the proportion of global symptom responders will be significantly larger at 6 months after cessation of therapy, compared with the placebo group.
2. Acceleration of solid gastric emptying, reduction of postprandial satiation and enhanced gastric volume change with a meal on antidepressant therapy will be significant positive predictors of beneficial short and long-term outcome in functional dyspepsia. Conversely, negative predictors of outcome will be slowed gastric emptying, increased postprandial satiation and reduced postprandial proximal gastric volume change.
3. The heterotrimeric G protein GNβ3 CC polymorphism will predict a significantly better symptom response to escitalopram and amitriptyline than TT or TC, and the serotonin transporter long homozygous polymorphism will predict a significantly poorer symptom response to the SSRI and amitriptyline.

In a parallel group, double blind, randomized, placebo-controlled adequately powered three-arm multi-center trial, the aims of the present study are to:
1. Determine whether antidepressant therapy is more efficacious than placebo in relief of the symptoms of functional dyspepsia, adjusting for psychological and psychiatric co-morbidity. We will also determine if antidepressant therapy reduces disability, improves quality of life and influences clinical response over 6 months after ceasing medication.
2. Determine if gastric emptying (motor dysfunction) and the nutrient drink test (a test that assesses gastric hypersensitivity and/or gastric accommodation) is altered by antidepressant therapy with a tricyclic or SSRI, and whether subgroups with altered physiology are associated with treatment outcome. In a sub-study, we will directly determine if impaired gastric accommodation (by a novel validated non-invasive imaging method using ^{99m}Tc-SPECT) and the symptom response to a nutrient drink test is altered by an SSRI or tricyclic antidepressant.
3. Determine if polymorphisms of GNβ3 and the serotonin reuptake transporter predict outcome in functional dyspepsia patients receiving a tricyclic antidepressant or SSRI therapy.

Although widely used in functional dyspepsia, the use of antidepressants is not evidence based. There have been no adequate randomized controlled trials with tricyclics or SSRI's in functional dyspepsia, but uncontrolled studies support their potential usefulness. There have not been any trials directly comparing tricyclics with SSRIs in any functional GI disorder, and there have been no long-term follow-up studies post antidepressant therapy in any of the functional GI disorders. In particular, whether prolonged exposure to antidepressants modifies the subsequent natural history of functional dyspepsia is unknown, but has major management implications.

I. Background and Significance

In the U.S., up to one in four people have symptoms suggestive of functional dyspepsia (1-3). About a quarter of these people seek medical assistance (2-4); functional dyspepsia and irritable bowel syndrome (IBS) account for over half of all GI consultations in the U.S. and remain the most frequent GI problems in primary care (2-4). In addition to substantially impairing quality of life (24), health care costs for functional dyspepsia have been calculated to be enormous, conservatively exceeding several billion dollars annually in the USA including billions of dollars for dyspepsia drugs (2,5,6). In a population-based study in Olmsted County, MN, 25% had dyspepsia in the prior year (1): a retrospective chart review of this cohort showed that over a 20 year period, 46% had undergone a physician consultation for dyspepsia and of these 91% received a prescription medicine; 18% had received a psychotropic medication (Locke, Talley et al: in preparation). The epidemiology of functional dyspepsia in African-Americans and Hispanics, and the response to treatment of different ethnic groups in the USA has been virtually ignored (2).

Functional dyspepsia is currently considered to be a bio-psychosocial disorder with disturbances of motor function, heightened visceral sensitivity, and possibly a central nervous system disturbance (7,8). Psychosocial factors can alter motility and/or enhance sensation and influence the timing of patients' presentation to physicians (7,8). The current proposal addresses the efficacy of drugs that act at the level of the peripheral gut and central nervous system, and the impact that physiology, psychological disturbance and genetic variation may play.

Currently, the treatment of functional dyspepsia is considered unsatisfactory and, for clinicians, frustrating because outcomes are heterogeneous (7,8). Standard treatment includes dietary advice of no established value and peripherally active pharmacological treatment including antisecretory agents (H_2 blockers, proton pump inhibitors) and prokinetics. In systematic reviews of the available therapies, it has been concluded that the only drugs established to be better than placebo in functional dyspepsia are possibly antisecretory and prokinetic agents (9-11). However, a Cochrane meta-analysis also suggested that the positive cisapride data may simply reflect publication bias, based on a funnel plot (9). Of the prokinetics, only metoclopramide is available in the USA since the withdrawal of cisapride and side effects limit its use; tegaserod, a serotonin type 4 receptor agonist, was of limited efficacy in a Phase II functional dyspepsia trial, but this may reflect the dosing choices made (12).
Many patients with dyspepsia turn to alternative therapies of totally unproven value (13). Non-pharmacological treatments have also been tested but only in a very limited fashion; hypnotherapy was superior to standard care in a recent single center study but is not widely available (14). A systematic review concluded that psychological therapy in functional dyspepsia is not of established value because of limited data (15). Psychological treatment also represents a labor intensive and costly approach. The lack of effective management in functional dyspepsia is likely to promote repeated medical consultation and its associated costs, as well as substantial amounts of time lost from work (2,5,7).

Psychosocial factors are potentially key modulators of experience, behavior and hence treatment outcome in functional dyspepsia but data here are limited. Patients with functional dyspepsia have been reported to have significantly higher levels of psychiatric illness than healthy controls (16-18) and patients with organic GI diseases (19). Others have confirmed higher levels of psychological distress in those with functional dyspepsia presenting in primary care (20) and in the general population (21), compared with healthy controls. Magni et al using standardized criteria found 67% of patients with functional dyspepsia met criteria for an anxiety disorder versus 20% for organic dyspepsia (17). Colgan found up to 57% of patients with functional upper abdominal pain were diagnosed with depression using standardized criteria, although the exact number of

patients with functional dyspepsia in this group is unclear (22). Porcellini et al compared scores on the Hospital Anxiety and Depression Scale and the Toronto Alexithymia scale between outpatients with a functional GI disorder (one-third met criteria for functional dyspepsia), 116 inflammatory bowel disease patients and controls; the functional GI disorder group was significantly more alexithymic controlling for depression (23). Using the MMPI, we found that only 32% of patients with functional dyspepsia could be correctly classified based on personality scores (24).

Antidepressants are used in the treatment of functional dyspepsia and IBS, usually in secondary or tertiary care, based on three propositions. First, antidepressants could reduce the severity of psychological symptoms, particularly anxiety and depression, which are thought to exacerbate the symptoms of functional dyspepsia, and may in some cases be etiologically linked to the syndrome although this is controversial (8,19-24). Second, antidepressants have central analgesic actions (25), and there is increasing evidence of central nervous system dysfunction at least in IBS (e.g. based on changes in cerebral blood flow)(26). Antidepressants reduce affective arousal and have sleep restorative actions (27,28). Thirdly, these drugs have been shown to have local pharmacological actions on the upper gut, specifically altering transit and gastric accommodation (see below).

The choice of antidepressant medication should depend upon the target symptoms, the overall clinical picture (including co-morbid disorders) and the side-effect profile. The therapeutic response and side effects of antidepressant medication tend to vary between individuals (27-29), suggesting that constitutional factors influence the response and pharmacogenomics may be of relevance.

There is accumulating evidence that low doses of a tricyclic are efficacious in IBS (see below) and other functional GI disorders (27,28,30,31), but the mechanisms remain obscure. Gorelick et al. compared the effects of amitriptyline on perception of cutaneous and gastrointestinal stimulation; cutaneous electrical stimulation and rectal and esophageal distension were performed before and after 21 days of double-blind 50 mg amitriptyline vs. placebo in healthy volunteers (32). Amitriptyline reduced perception of cutaneous stimulation but did not alter visceral perception or compliance. In non-cardiac chest pain, low dose imipramine has been demonstrated to significantly reduce the number of pain episodes (33,34). Moreover, the pain literature supports the concept that pain relief occurs with non-antidepressant doses of tricyclics (28,35). Limited uncontrolled data also suggest that low dose tricyclics including amitriptyline may be efficacious in functional nausea and vomiting (30).

Studies on the analgesic effects of SSRIs are promising (25,36). In a double-blind trial of 30 patients with non-cardiac chest pain, sertraline significantly reduced the pain versus placebo (37). Citalopram also significantly reduced pain and tended to improve well being in a controlled trial in fibromyalgia (38), of whom up to 50% have functional dyspepsia (39). In a physiological study in patients with IBS, a single intravenous dose of citalopram did not alter colonic sensitivity to distention (40); however, a subsequent 6-week treatment with citalopram was associated with a reduction in frequency and severity of abdominal pain and bloating, and lower global symptom scores (41) suggesting that the effect of this SSRI on IBS symptoms may be the result of central mechanisms. On the other hand, we found no effect of venlafaxine on colonic pressure thresholds although compliance was altered (42).

Meta-analyses support a benefit on chronic pain of the SSRI drug class (29,31,43), but whether any benefit represents a change in affect rather than analgesia is still debated. O'Malley et al. (29) concluded that both tricyclics and SSRIs are efficacious for various physical symptoms including pain and this was not correlated with the response of depression, but the evidence for the latter was very limited.

Gender differences in the response of depression to different antidepressant classes has been observed but not explained; recent data in a 12 week trial of depression suggest that females may respond better to an

SSRI and males to a tricyclic (44). It is unknown if such gender specific responses occur in functional dyspepsia, although females may respond better than males to the serotonin type 3 receptor antagonist alosetron and the serotonin type 4 receptor agonist tegaserod in IBS (45,46).

It is our clinical experience that both tricyclics in low dose and SSRI's at standard doses can be very effective in providing symptomatic relief in functional dyspepsia when treatment is prescribed for at least one month in women and men. Moreover, the symptom benefit appears to persist on stopping therapy for a prolonged period in some cases. However, very little trial data are available with tricyclics in functional dyspepsia (see below) and none with the SSRIs. There have not been any systematic trials comparing these two classes of compounds head-to-head in functional dyspepsia, and no long-term follow-up studies at all. Moreover, mechanisms for any benefit have not been established. We will investigate all of these key issues in this proposal.

Previous studies of antidepressants in functional dyspepsia are very limited

A meta-analysis by Jackson et al. of 12 randomized trials concluded that treatment of functional gastrointestinal disorders with tricyclic antidepressants appears to be effective in low dose (with the number needed to treat (NNT) being an impressive 3), but whether this improvement is independent of an effect of treatment on depression was uncertain (31). However, these were almost exclusively IBS trials, they generally were under-powered, and were often of poor methodological quality.

Clouse et al. evaluated 138 IBS patients with a series of antidepressant medications in an open study (28). They found that dosages of tricyclic antidepressants lower than those conventionally used to treat psychiatric disorders appeared to be efficacious in IBS. Notably, the gastrointestinal benefits were independent of changes in mood, and IBS patients with no psychopathology obtained symptom benefit on a low dose tricyclic antidepressant (28). However, placebo effects, spontaneous remission, and regression to the mean may all explain these observations. Drossman et al. randomized patients with IBS and chronic abdominal pain to desipramine versus placebo (n=216) (47). Desipramine 50 to 150 mg was prescribed in patients with moderate to severe symptoms; 73% responded to desipramine compared with 49% to placebo (per protocol analysis), and this benefit was not associated with preexisting depression; the response was not significantly different in those on 50 mg versus 100 or 150 mg.

In the only clinical trial to directly investigate functional dyspepsia, Mertz et al. randomized 7 patients to 4 wk of amitriptyline 50 mg taken at bedtime versus placebo (48). There was a 3-wk washout phase, followed by a cross-over to the alternate treatment. Perceptual sensitivity to gastric distention and sleep EEG were recorded at the end of each treatment period. Diaries of symptoms were maintained throughout. All patients reported significantly less severe gastrointestinal symptoms after 4 wk on amitriptyline. Five of 7 patients had evidence for altered perception of gastric balloon distension during placebo. However, the subjective symptom improvement on amitriptyline was not associated with a normalization of the perceptual responses to gastric distension in this small but important pilot study. In the Mertz trial (48), 71% (5 of 7) reported global symptom improvement versus 28% (2 of 7) on placebo; this translates into an impressive NNT of 2.

Tanum and Malt used the tetracyclic antidepressant, Mianserin, at a dose of 120mg daily versus placebo in 49 patients with various functional GI disorders who failed a placebo run-in (49). They found that patients taking mianserin compared with placebo reported significantly less abdominal pain and functional disability, therapy was efficacious regardless of the type of functional disorder, and benefit persisted 4 weeks after tapering (49). However, several study design issues seriously limit the studies interpretability, and side effects were problematic. In a separate study, the same investigators reported in 48 patients with a functional GI disorder that moderate to high neuroticism and marked concealed aggressiveness predicted a poor response to

mianserin (50). In the absence of other data, it can be concluded that use of tricyclics in functional dyspepsia is not evidence based but amitriptyline is arguably most promising.

Uncontrolled trials suggest that SSRI's in standard dose are efficacious in IBS (28) and small controlled trials have produced positive findings in IBS for improvement of pain (40,51). There have been no published randomized controlled trials with SSRIs in functional dyspepsia, despite their reasonably widespread use in specialist clinical practice for patients with functional dyspepsia. Finally, the impact of psychological co-morbidity on treatment response in functional dyspepsia remains essentially unknown, but the issue of whether changes in psychological disturbances predict therapeutic outcome is important.

Using prescription sequence symmetry analysis in a large database of one million prescriptions in Denmark, Bytzer and Hallas reported that antidepressant use was associated with a significant protective effect against functional dyspepsia, but the type of antidepressant was not specified (52).

The link with psychological and psychiatric co-morbidity suggests that a peripherally acting agent alone is unlikely to provide substantial relief in those with functional dyspepsia presenting for care, and supports the concept of using a centrally acting agent. However, the peripheral effects of such agents on gastric function could also conceivably contribute positively or negatively to the response to therapy (see below). The AGA, World Congresses of Gastroenterology and Rome Committee guidelines on dyspepsia management include antidepressants as a recommended treatment in those failing first line therapies (7,8) but this is not evidence based.

Symptom subgroups in functional dyspepsia and treatment outcome

Physiological measurements are needed to complement the characterization of functional dyspepsia phenotypes and to assess the response to therapy. It has been proposed that functional dyspepsia can be subdivided into symptom subgroups in order to reduce heterogeneity (1,8). However, the current classification into ulcer-like and dysmotility-like dyspepsia is controversial. The best support comes from a trial of 1248 patients randomized to a proton pump inhibitor (PPI) or placebo in functional dyspepsia; those with predominant epigastric pain (ulcer-like) were significantly more likely to have relief of dyspepsia on PPI over placebo, but no therapeutic benefit was observed in those with predominant early satiety or fullness (dysmotility-like dyspepsia) (53). However, in a recent study of Chinese patients these observations were not confirmed (54). We anticipate most patients in a trial would fall into one or other of these subgroups based on using symptom predominance (53). Evidence that symptom sub-grouping predicts treatment response in functional dyspepsia is lacking but if confirmed would have important implications for clinical practice.

Delayed gastric emptying, gastric hypersensitivity and failure of fundic relaxation.

Three physiological mechanisms are considered of major importance in functional dyspepsia:

- Delayed gastric emptying (40%) (55,56)
- Failure of fundic accommodation to a meal (40%) (57)
- Gastric and duodenal hypersensitivity (33%) (58,59)

However, the relationship of symptoms to these abnormalities is unclear and the abnormalities can overlap (one or more occur in 60-80% of patients); it is also unknown if antidepressants affect these abnormalities, and whether pharmacologically altering gastric dysfunction correlates with improved symptoms in functional dyspepsia. Stanghellini et al. in 343 Italian patients reported that female sex, relevant and severe postprandial fullness and severe vomiting were independently associated with delayed gastric emptying of solids (55). In a separate study of 483 patients, the same Italian group identified distinct subgroups: one characterized by predominant epigastric pain, male gender and normal gastric emptying, and a second

characterized by predominant non-painful symptoms, female gender, a high frequency of associated IBS and delayed gastric emptying (60). However, other studies have failed to identify a definite symptom profile associated with delayed gastric emptying (61).

Tack et al. recently reported in 160 patients with functional dyspepsia that one third had gastric hypersensitivity and this was associated with increased postprandial pain as well as belching and weight loss (59). These results require confirmation.

Tack et al. in another study of 40 patients with functional dyspepsia identified impaired gastric accommodation to a meal in 40%, and this abnormality was associated with early satiety and weight loss but not with hypersensitivity to gastric distention, presence of H. pylori, or delayed gastric emptying (57). Boeckxstaens et al failed to replicate these findings; there was no clear symptom profile that was associated with impaired fundic relaxation (62).

Physiological abnormalities as predictors of symptom outcome in functional dyspepsia

It is controversial whether the identified gastric function abnormalities are of primary importance in symptom generation. If they are relevant, then correcting the abnormality should relieve symptoms. There is, however, currently a lack of convincing data that physiological subgroups predict treatment response. Van Zanten et al. concluded in a meta-analysis that there were insufficient data to determine whether there is a relationship between improvement in gastric emptying and response to treatment with the prokinetics cisapride or domperidone (10). Tack observed that the $5HT_1$ agonist sumatriptan restored gastric accommodation, and significantly improved meal-induced satiety, suggesting drugs that modulate fundic relaxation may have a therapeutic place in functional dyspepsia (57,63). However, limited other studies have not replicated these observations (62).

Antidepressants may alter upper GI tract function in healthy volunteers, but limited data are available (64-68) and the relevance to symptom outcomes equally unclear, as patients with functional dyspepsia have not been evaluated. Gorard et al. reported that imipramine (100 mg daily) prolonged orocecal transit times in controls and diarrhea-predominant IBS patients, while paroxetine (30 mg daily) decreased orocecal transit time in both IBS sufferers and controls (64). Both paroxetine and imipramine altered gut transit time before any change in mood was reported (64). There are no published data on the effects of tricyclics on fundic relaxation, but theoretically gastric accommodation could be enhanced by an anticholinergic effect of this drug class, and here amitriptyline may be of most interest.

Author	Antidepressant	Motor effect evaluated in healthy volunteers
Gorard et al. (64)	Imipramine	Slowed orocecal transit
Gorard et al. (64)	Paroxetine	Accelerated orocecal transit
Ladabaum (65)	Sertraline	No effect on gastric compliance
Tack et al. (66)	Paroxetine	Postprandial fundic relaxation
Chial et al. (67)	Paroxetine	No effect on gastric accommodation; accelerated small bowel transit
Chial et al. (67)	Venlafaxine	Increased gastric accommodation
Broekaert et al. (68)	Citalopram	Reduced esophageal sensitivity; gastric function not tested

Ladabaum and Glidden reported in 10 healthy volunteers, the SSRI sertraline had no effect on gastric sensitivity or compliance, or somatic pain tolerance, but this was a crossover study, and patients

were not assessed (65). Other data suggest that citalopram reduces esophageal sensitivity (68). In a randomized, double-blind physiological study (n=51), we evaluated buspirone, a 5-HT1a receptor agonist; paroxetine, an SSRI 20 mg daily; venlafaxine-XR, a selective serotonin and norepinephrine reuptake inhibitor, 75 mg daily; or placebo for 11 days (67). No effects on gastric emptying were identified with any agent but small bowel transit of a solid meal was accelerated by paroxetine. There was a trend for paroxetine but not venlafaxine to decrease postprandial aggregate symptom scores, as well as nausea, fullness and pain; while venlafaxine-XR increased the postprandial change in gastric volume, paroxetine did not (67). Tack et al. on the other hand studied 12 healthy volunteers by a gastric barostat study on two occasions, after pre-treatment with placebo or paroxetine 20 mg/day (66). Pre-treatment with paroxetine 20mg daily did not alter thresholds for perception or discomfort, either during isobaric or isovolumetric distensions. However, paroxetine significantly enhanced the amplitude of meal-induced fundus relaxation (66). The findings overall suggest that release of 5-HT, probably at the level of the enteric nervous system, may be involved in the control of the accommodation reflex in health, but convincing evidence is still needed. Hence, SSRIs as a drug class may relieve symptoms in patients with impaired postprandial fundus relaxation by relaxing the gastric fundus via the effects of increased 5HT (possibly acting via 5HT1a receptors). However studies in disease, and in particular in functional dyspepsia, are lacking. It has been our clinical impression that those with normal gastric emptying respond preferentially to the tricyclic antidepressants whereas those with impaired ability to eat a normal meal, implying fundic disaccommodation, respond best to the SSRIs.

Until recently, it was impractical to assess stomach accommodation and sensation because of the need for intubation. A unique noninvasive SPECT method developed and validated in our laboratory (see preliminary data) now permits the measurement of gastric accommodation (69,70). Gastric hypersensitivity can also indirectly and non-invasively be assessed using a nutrient drink test, with standard methods and endpoints (71). These techniques all developed and standardized in our laboratory, will be applied in the proposed clinical trial to identify physiological subgroups.

Functional dyspepsia and familial aggregation

We have observed in 643 subjects randomly selected from Olmsted County, MN, that among those reporting a first-degree relative with abdominal pain or bowel problems, there was a significantly increased risk of having dyspepsia (OR, 1.8; 95% CI, 1.05-3.0) but not constipation, diarrhea, or gastroesophageal reflux (72); the reporting of a spouse with abdominal pain or bowel problems was not associated with any of these disorders (72). We postulate that the familial associations represent a genetic factor, although other explanations are possible. Recent twin studies assessing dyspepsia support the genetic hypothesis, and concur with data in IBS (73-75). Our study will not address if genetic factors are linked with functional dyspepsia or psychiatric disease directly, or independently predispose to both. Rather, we are interested here in whether genetic factors partly explain the presumed wide variability in response to different classes of antidepressant therapy in functional dyspepsia.

Response to antidepressants and pharmacogenetics.

The response to antidepressants is heterogeneous in clinical practice, and genetic variation may explain this at least in part (76). Two specific polymorphisms that modulate serotonergic and adrenergic pathways are of particular interest based on the actions of the two drug classes to be tested and preliminary data (see below). Tricyclics variably affect multiple receptors, while the SSRIs have more selective effects on serotonergic reuptake (transporter) functions. We shall therefore explore the influence of variation in two candidate genetic mechanisms that control serotonergic function (transporter) and translation of multiple relevant ligand-receptor functions (heterotrimeric G protein).

It is recognized that a polymorphic site in the promoter region for the serotonin transporter protein is associated with differences in serotonin transporter (5HTTLPR) gene expression (77,78). Lesch et al. showed in

human lymphoblasts expressing the 3 common SERT genotypes that there was higher 5HT transport and higher messenger RNA for the transporter protein in the long homozygous (LL) compared with the other cells (SS,LS)(78). We hypothesize that the long homozygous SERT polymorphism will reduce antidepressant efficacy in the gut because more 5HT will be removed by the transporter. Conversely, the short homozygous polymorphism will increase the drug's efficacy as more 5HT will be available.

The functional significance of these genetic polymorphisms is supported by a recent pharmacogenetic study in IBS from our group. We have shown that serotonin-transporter homozygotes for the wild-type (long/long LL) alleles had slower colonic transit times and were more likely to respond to alosetron, a $5HT_3$ receptor antagonist (79). This was hypothesized to occur because the long SERT polymorphism leads to a lower synaptic concentration of 5HT that needed to be competitively inhibited by alosetron. No pharmacogenetic data are available in functional dyspepsia.

Other 5HTTLPR polymorphisms may be relevant but remain to be evaluated in functional dyspepsia and IBS. For example, patients with depression may have reduced responsiveness to SSRIs with the short allele of a variable number of tandem repeats (VNTR) polymorphism (80). It is unknown if the polymorphism data has implications for SSRI therapy in functional dyspepsia.

The heterotrimeric G-proteins are essential for stimulus-response coupling of receptors which are linked to intracellular effector systems such as the adenyl cyclases, the phosphoinositide system, as well as various protein kinases and transcription factors (81-84). A specific G β3-gene polymorphism (C825) have been linked to depression (85,86), altered immunological responses (87,88) and disturbed alpha 2-adrenoreceptor function (84). Altered adrenergic receptor function may play a role in disturbed sensory gut function (89). The 5HT1 and 5HT4 receptor are G protein coupled, while the 5HT3 receptor is a ligand operated ion gated channel (45,46); drugs that stimulate 5HT4 receptors (prokinetics e.g. tegaserod, cisapride) or 5HT1a receptors (fundic accommodation relaxing agents e.g. sumatriptan) probably have some efficacy in functional dyspepsia (10,12,63). Antidepressants affect multiple receptors; approximately 80 % of all known membrane receptors transduce their signals via heterotrimeric G-proteins, including serotonergic and adrenergic receptors.

5HT (serotonin) and NE (norepinephrine) release: activation of G proteins post-synaptically varies with C825Tgenotype in GNβ3. The heterotrimeric G proteins are essential for stimulus-response coupling of receptors: TT is associated with increased signal transduction; CC with reduced signal transduction.

Heterotrimeric G-proteins are composed of different alpha, beta and gamma subunit isoforms, the beta-gamma subunit forming a functional monomer. On receptor activation, both alpha and beta-gamma subunits dissociate from the receptor and in turn modulate a large variety of intracellular effector systems. Thus, heterotrimeric G-protein dysfunction could represent a potential block for intracellular signal transduction.

A common C825T polymorphism has been described in the gene GNβ3 which encodes the beta 3 subunit of the G-protein. This polymorphism gives rise to three possible genotypes, i.e. CC, TC and TT. The 825T allele within the TC or TT genotype is associated with different splicing of the gene and the formation of a truncated but functionally active splice variant. In general, the 825T allele is predictive of enhanced G protein activation and, thereby, increased cellular or physiological responses (84). Homozygous 825C allele carriers (CC genotype) form only minute amounts of the β3 splice variant and thus are characterized by diminished signal transduction responses. GNβ3 is an important translational arm of various serotonergic and adrenergic receptors and hence it is important to assess pharmacological effects and their modulation by different GNβ3 genotypes. Pharmacogenetic studies have shown that GNβ3 polymorphisms affect therapeutic response in other smooth muscle, such as the vascular system to anti-hypertensives (90). It is also conceivable that G protein polymorphisms could lead to specific alterations in gastric function that in turn leads to symptoms in functional dyspepsia.

We have pilot data that the GNβ3 CC polymorphism is associated with functional dyspepsia (see pilot studies below). There are race differences; the table shows the prevalence of GNβ3 polymorphisms in healthy Whites and Blacks reported in the literature (91). We therefore hypothesize that we will observe less responsiveness to antidepressants in Blacks than Whites with functional dyspepsia.

Reference	Blacks (USA)			Whites (world)		
	TT	TC	CC	TT	TC	CC
Siffert et al. 1999 (91)	51%	42%	7%	11%	43.5%	45.5%

We will specifically be able to assess the CC response to therapy. Associations between responses to therapy in functional dyspepsia based on heterotrimeric G protein or serotonergic genotypes are, however, unknown.

Summary of Background and Significance

Functional dyspepsia is a chronic, heterogeneous disorder that seriously impairs quality of life and is very costly (2,7,8). Treatment currently is very unsatisfactory and the outcome is variable, probably in part because the disorder is heterogeneous (7,8). Antidepressants, whilst often used, are of uncertain efficacy. Information on the therapeutic benefits and whether subgroups of functional dyspepsia can be identified who would benefit most is of major clinical relevance. The pharmaceutical industry is largely not interested in testing antidepressants in the functional GI disorders because: i) many drugs are off patent or have a short patent life remaining, ii) standard antidepressants are relatively inexpensive, and iii) it is not in their interests to compare a newer antidepressant (e.g. an SSRI) to a generic tricyclic in a head-to-head comparison, as similar efficacy may be demonstrated.

II. Progress Report and Preliminary Studies

1. Development and testing of new outcome measures in functional dyspepsia

We are leaders in the development of outcome measures in the functional GI disorders. We have specifically developed and tested in the USA valid symptom diagnostic tools (e.g. Bowel Disease Questionnaire) (92), assessed new outcome measures for responsiveness (e.g. GSRS) (93) and developed disease specific quality of life measures (Nepean Dyspepsia Index) (94-96) for trials in functional dyspepsia; these will be applied in this grant.

2. Development of noninvasive methods to evaluate gastric accommodation

The role of gastric dysfunction in predicting treatment outcome in functional dyspepsia is incompletely understood. We propose to fill this key research gap in this study. In particular, impaired gastric accommodation is associated with early satiety and weight loss (57,63) but accommodation has not been evaluated in trials of therapy in functional dyspepsia because of the need for invasive gastric measurements using a barostat balloon There have been no trials which have directly compared tricyclics and SSRIs for the treatment of functional dyspepsia, and no randomized controlled trials of the SSRIs on their own. We have developed a noninvasive technique to measure gastric volume and hence accommodation (69,70,97), and plan to apply this novel technique in the proposed trial.

(i) Method development: we use i.v. ^{99m} Tc pertechnetate, single photon emission computed tomography (SPECT) imaging and adaptation of the ANALYZE ^{TM} program for volume rendering, three-dimensional reconstruction and estimation of volumes. When using the SMV gamma camera for Spect acquisition, each set of scans take approximately 16 minutes/ acquisition. Scan acquisition using the Siemens MSII requires approximately 12 minutes per scan.

(ii) In a healthy volunteer study (n=73) we established range of stomach volumes in health (98):

Volumes, ml	Whole stomach, fasting	Whole stomach, fed	Prox stomach, fasting	Prox. stomach, fed
All, n =73	213 ± 6	698 ± 12	112 ± 5	407 ± 15
Males, n =25	215 ± 11	744 ± 20	126 ± 11	474 ± 26
Females, n=48	211 ± 7	675 ± 14	105 ± 6	374 ± 17

(iii) The SPECT method to measure gastric volume has been validated in our laboratory by comparison with simultaneous measurement of gastric volume change with a barostatic balloon (98).
(iv) We have confirmed the sensitivity of the method to detect predictable pharmacological effects (e.g. a nitrate and a motilide, erythromycin) (69), confirming results obtained with the barostat technique (99).
(v) We have evaluated the gastric accommodation response (ratio postprandial/ fasting volume) in 32 patients with postprandial upper gastrointestinal symptoms (70) consistent with functional dyspepsia vs. 20 controls (Fig.2): we found that ~40% of dyspepsia patients had reduced gastric accommodation, confirming results from Europe and showing it is appropriate to apply this new technique in the proposed trial.

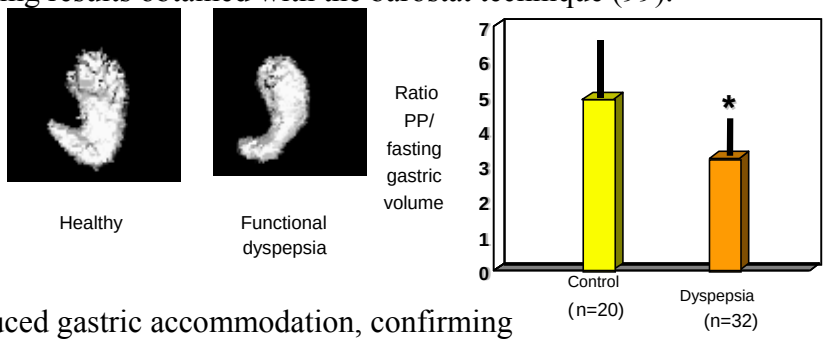

Fig 2. *Postprandial (PP)volume reduced in dyspepsia*

3. Pilot data on effects of tricyclics and an SSRI on gastric function and meal induced symptoms Since the last submission, we have collected new pilot data to refine the planned clinical trial.
i) Pilot study of desipramine versus escitalopram and placebo on postprandial symptoms in healthy volunteers
Desipramine and escitalopram are antidepressant medications that have been used to treat functional gastrointestinal disorders but the effect of these medications on post-prandial symptoms measured in a controlled experimental design remains unstudied. We hypothesized that desipramine and escitalopram would enhance maximum tolerated volume of food ingested and decrease postprandial symptoms. We compared the 11 day effects of desipramine, escitalopram and placebo on maximum tolerated volume and postprandial symptoms in healthy participants (n=45). We conducted a randomized, parallel group, single dose, double blinded, placebo-controlled study to evaluate the effects of desipramine and escitalopram on gastric function along with symptoms in healthy participants. All participants underwent an assessment of baseline symptoms and a nutrient drink test. Participants were then randomized to 11 days of treatment with desipramine (50 mg p.o. q.d), escitalopram (10 mg p.o. q.d.) or placebo. The nutrient drink test along with an assessment of symptoms was repeated at the end of the study. Based on data acquired using the same methods in the laboratory, the sample size of 15 subjects per group provided 80% power to detect approximately 20-80% changes in the primary and secondary endpoints of the study (that is, maximum tolerated volume, aggregate and individual post-prandial symptom score, and change in gastrointestinal symptoms from baseline). There were no significant differences observed in the baseline characteristics of the groups. The maximum tolerated volumes were not significantly different for desipramine (1136 mls, (SD 478)), escitalopram (1198 mls (SD 422)) and placebo (1231 mls (SD 318), Fig3).

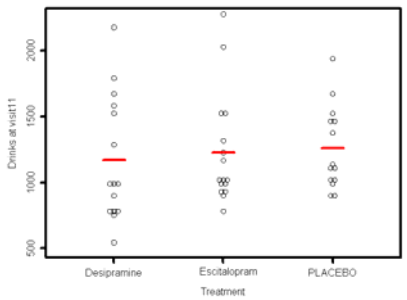

Figure3. Maximum tolerated

Similarly, the total symptom scores were not significantly different on day 11 for desipramine (mean 141.1 (SD 59.9)), escitalopram (151.3 (SD 56.5)) and placebo (mean 103.3 (SD 47.7)). Notably, these were not patients with functional dyspepsia, adequate relief of symptoms (the now accepted primary endpoint for these trials) could not be assessed, and the study duration was short.

ii) Randomized dose response study of amitriptyline versus placebo in healthy volunteers

Healthy volunteers (n=41) were randomized to amitriptyline (25mg, n=14; or 50mg n=13) or placebo (n=14) for 2 wks. Measured were gastric emptying by scintigraphy, gastric accommodation (SPECT), and Ensure volumes ingested as well as symptoms induced by the nutrient drink test. We found that nausea was significantly reduced on active therapy in an apparent dose dependent manner (placebo 2.1 (0.8, 5.3); 25 mg amitriptyline 0.9 (0.3, 2.3); 50 mg amitriptyline 0.0 (0.0, 0.5) (P=0.009). There was also a trend for the total symptom score to improve. There was no association between dose and gastric volume ratio or volume of Ensure ingested. Gastric emptying was not significantly delayed at 2 or 4 hours on amitriptyline 25mg or 50mg.

In summary, amitriptyline reduced postprandial symptoms score (especially nausea) after a nutrient challenge without any significant change in gastric volumes or gastric emptying.

4. Heterotrimeric G protein and other polymorphisms

i) Heterotrimeric G protein polymorphisms and functional dyspepsia

We have observed a potential association between a polymorphism on the β 3 subunit of the G protein (GNβ3 C825T) and functional dyspepsia; the results have now been published in full in Gastroenterology (100). In study A, abdominal symptoms were assessed in 67 patients with unexplained, upper abdominal symptoms and 259 consecutive blood donors with and without abdominal symptoms. In study B, a further 56 patients with functional dyspepsia and 112 age- and sex-matched healthy controls from a blood donor population study were evaluated. Genomic DNA was isolated from buccal swabs and genotyping of the C825T polymorphisms was performed by polymerase chain reaction and restriction analysis. In the blood donors with no abdominal symptoms in study A (controls, n = 161), genotype distribution was 17 TT, 77 TC, and 67 CC. In blood donors and patients with unexplained abdominal symptoms, genotype distribution was 22 TT, 54 TC, and 89 CC (P = 0.007 vs. controls). In study B, the genotype distribution in functional dyspepsia patients was 4 TT, 18 CT, and 34 CC compared with 4 TT, 62 CT, and 46 CC in the controls (P < 0.02). Combining studies A and B, the odds ratio (OR) adjusted for age and sex for upper abdominal symptoms associated with the CC genotype was 2.2 (95% confidence interval [CI]: 1.4-3.3), compared with subjects with TC and TT genotype carrying an allele. These novel data suggest that the CC polymorphism is associated with functional dyspepsia, a conclusion considerably strengthened by independent confirmation in two different patient cohorts. Furthermore, in another independent study, we have now confirmed these observations; these data were presented at DDW in 2004. We studied consecutive patients with functional dyspepsia, irritable bowel disorder (IBS) or a combination of both. The most dominant symptom complex (IBS, dysmotility-ulcer-dyspepsia) was assessed. As controls, data from 2727 healthy blood donors were used. Patients were categorized based upon the most dominant (bothersome) symptom complex; 262 patients had functional dyspepsia and 174 patients were diagnosed having IBS (88 had simultaneously IBS and functional dyspepsia). Overall, the GNβ3 CC genotype was significantly more prevalent in patients with functional dyspepsia and/or IBS (55.2, 95% 50.0-60.4) compared to blood donors (47.4, 95% CI 45.6-49.3). The relative risk attributable to the GNβ3 CC genotype for predominant ulcer-type symptoms was 1.73 (95% CI 1.2-2.6). The association between the GNβ3 CC genotype and dysmotility-type dyspepsia and IBS were not significant (1.3, 95% CI 0.94-1.89 and 1.2, 95% CI 0.94-1.6). We speculate that the GNβ3 CC genotype plays a role in the processing of visceral afferents for pain (101).

ii) Heterotrimeric G protein polymorphisms, functional dyspepsia subgroup and co-morbid psychiatric disease

To evaluate the association between psychiatric co-morbidity, G- protein genotype and the clinical presentation of patients with functional dyspepsia, we evaluated another 240 patients (147 female). Presence of anxiety and depressive disorders was based on clinical diagnosis by a trained physician blinded to GNβ3 status. Patients were classified based upon the predominant symptom pattern into ulcer- and dysmotility- like functional dyspepsia, or IBS. Logistic regression analysis adjusting for age identified GNβ3 CC status and male gender as independent risk factors for ulcer-like dyspepsia. Independent risk factors for dysmotility- like dyspepsia were GNβ3 CC, anxiety and depression. BMI was adjusted for in the analyses because it is associated with GNβ3. Based on this and our other pilot data, we conclude that ulcer-like functional dyspepsia is associated with GNβ3 CC genotype. We will adjust for potential confounders including race and body mass index (BMI) in our planned analyses in the trial.

iii) Heterotrimeric G protein polymorphisms, functional dyspepsia and response to therapy

We have new pilot data on the association of GNβ3 with response to therapy in FD (102). We recruited 80 patients with chronic or relapsing symptoms (> 5 years) that were referred to a tertiary referral center for evaluation and treatment of nonresponsive symptoms. All patients had predominantly upper abdominal symptoms with the final diagnosis of functional dyspepsia after extensive diagnostic work up. Presence of anxiety and depressive disorders were clinically judged by a trained physician. All patients were treated with whatever treatment the clinician chose, utilizing acid suppression, prokinetics or antidepressants. After 12 months, patients were categorized independently based upon their judgment as responders or nonresponders. After 12 months, 23 out of 80 patients were categorized as responders (R+) while 57 patients were categorized as non-responders (NR). The GNβ3 CC polymorphism was found in 56% of patients and was univariately associated with R+ ($p<0.02$), while dysmotility-type ($p<0.02$), concomitant IBS-symptoms ($p<0.05$) and the presence of an anxiety disorders ($p<0.05$) were associated with NR. Logistic regression analysis revealed a significant ($p<0.05$) association between the R+ and the CC polymorphisms while there was a trend for a negative association between dysmotility-symptoms. Using CC genotype (versus TT and TC) as the test, and response to therapy as the comparative standard, the sensitivity in this pilot study was 78% and the specificity was 53% (odds ratio for response 4.0, 95% CI 1.3, 12.2). The negative predictive value was therefore excellent, at 86%. Molecular variants of the GNβ3 that modulate receptor cell-coupling may independently influence outcome and response to medical therapy in functional dyspepsia (102).

iv) Other polymorphisms

Norepinephrine transporter (NET) polymorphisms may be of relevance theoretically in terms of antidepressant response. However, we have tested this hypothesis in 100 patients with IBS or chronic abdominal pain and 20 ethnically matched healthy controls; no NET polymorphisms were identified (103). A number of other putative polymorphisms could be tested but there remains insufficient justification to do so based on the current literature and our pilot data.

5. Optimization of molecular assays for candidate genes

DNA Bank

We have stored DNA samples extracted from whole blood buffy coat from patients with IBS, identified from the Rochester Epidemiology project. Our IBS patient population is among the best characterized in the country from a phenotypic perspective. We plan to develop a similar DNA bank for functional dyspepsia from this trial; storage of genetic material from these patients constitutes a UNIQUE resource for the current and future studies of the role of genotype on the response to pharmacotherapy in functional dyspepsia. Other studies will be spawned from this phenotype and DNA bank.

Detection of Polymorphisms

The selection of 2 candidate polymorphisms (5HTTLPR and GNβ3) is based on our pilot data and published data. It is our hypothesis that these polymorphisms may play a role in the response of patients with functional dyspepsia to antidepressants. We are developing assays for VNTR and other relevant polymorphisms. In future proposals, we will consider testing for other polymorphisms depending on the results.

Establishment of a functional dyspepsia patient cohort

We have established a cohort of patients living within a 200 mile radius of the Mayo Clinic in Rochester (n=600). To date, 214 patients with upper GI symptoms, the majority diagnosed as having functional dyspepsia, have been studied by SPECT. Hence, we anticipate recruitment should not pose a problem for studying gastric accommodation at the Rochester site. The other sites see large numbers of patients with functional dyspepsia and also anticipate no problems with rapid recruitment into the clinical trial (see co-investigator letters).

Summary and Gaps in Current Knowledge

It is unknown why the response to therapy in functional dyspepsia is variable, but differences among subgroups based on physiology and gene encoding relevant translational mechanisms may reasonably contribute to a variable clinical response of functional dyspepsia to antidepressant therapy. By characterizing the phenotypic expression of gut manifestations in functional dyspepsia at baseline and in response to antidepressants, we will characterize the pharmacogenetic responses to tricyclics and selective serotonin reuptake inhibitors in this disorder for the first time.

I. Research Design and Methods

Trial design and management

We have been involved in directing the Rome Multinational Working Team process (Rome I, II and III) which systematically reviewed the methodological limitations of the clinical trials in functional gastrointestinal disease, and has provided recommendations for future trial design (104). An overarching principle was that the trial should reflect the real world, include standard medical care, and apply rigorous and comprehensive outcome evaluations. The Rome process has set the standard for clinical trials in the field, and the recommendations will be applied here.

In our clinical practice, we have had extensive experience in using both the tricyclic antidepressants and the SSRIs for patients with functional GI disorders including functional dyspepsia, with and without co-morbid anxiety and depression. We have a demonstrated ability to recruit patients with various functional GI disorders and undertake long term follow-up in multicenter trials in the USA and internationally (13,53,93,105-118). The GI Division at Mayo Clinic group in close collaboration with Biostatistics has substantial experience with coordinating large multicenter trials sponsored by NIH (e.g. currently RO1 DK56924-02 and RO1 DK58369-02) and industry (currently 26 multicenter trials).

We will conduct a prospective, randomized, double-blind parallel group controlled trial, comparing a low dose tricyclic antidepressant with an SSRI and identical placebo. Amitriptyline hydrochloride is a tricyclic antidepressant; it has a half-life of 9-25 hours. We chose amitriptyline because of our new pilot data demonstrating changes on postprandial symptoms in healthy volunteers after a nutrient drink test at the dose planned. In addition, there is one small crossover trial of amitriptyline versus placebo that provides further albeit very limited evidence in FD that amitriptyline has efficacy (48). Moreover, it is our strong clinical impression the tricyclics are effective in dyspepsia at the dose to be used (50mg). We showed in our pilot studies that amitriptyline at the dose planned has no effect on impairing gastric emptying and so should not aggravate dyspepsia in those with delayed emptying. Its effects on reuptake of serotonin also make amitriptyline

of interest in terms of the planned pharmacogenetic studies of SERT polymorphisms. At the dose planned, the side effect profile should also be very acceptable.

Escitalopram (S-citalopram) is the new S-enantiomer of citalopram and binds with high affinity to the human serotonin transporter. It has a half-life of 27 - 32 h, leading to once-daily dosing (10mg). It likely has a low potential for drug-drug interactions because it has negligible effects on cytochrome P450 drug-metabolizing enzymes in vitro. There has been a low rate of discontinuation due to adverse events, no different from placebo in clinical trials. The most common side effects associated with escitalopram which occurred at a rate greater than placebo have been diarrhea, nausea, insomnia, ejaculation disorder, dry mouth and somnolence. We chose escitalopram based on our strong clinical impression that citalopram and escitalopram are effective in dyspepsia and because it is not a racemic mixture like fluoxetine; it is also one of the most selective SSRI's, which should enhance interpretation of the physiological and pharmacogenetic studies. Sertraline has been shown to have no effect on gastric compliance (67). Venlafaxine is both a serotonin and norepinephrine reuptake inhibitor and so was not considered as suitable either because we wished to focus on serotonergic mechanisms; venlafaxine has also failed to alter colonic sensory thresholds in IBS (42), unlike citalopram (40). We showed in our pilot study that escitalopram did not alter gastric volume ingestion in healthy volunteers, but whether it may alter gastric accommodation in health, or symptoms and gastric function in functional dyspepsia, has not been tested. We consider it likely based on our clinical experience the drug will provide global relief of symptoms in functional dyspepsia; this endpoint cannot be tested in a healthy volunteer study. Citalopram has also shown promising efficacy in IBS (41) and fibromyalgia (38) as well as in reducing esophageal sensitivity (68). Forest Pharmaceuticals have agreed to provide escitalopram and identical placebo without charge for this trial.

All subjects in the study will be provided with the same baseline level of psychological support encompassing reassurance and education. The trial will be conducted according to the Rome guidelines for clinical trials for the functional GI disorders published in 1999 (104), and will also adhere to the updated CONSORT reporting guidelines (119).

Subjects: Patients with functional dyspepsia

Patients will be enrolled at GI clinics in six sites to provide a geographically and ethnically diverse population. All participants will complete validated self report questionnaires at baseline that we have used extensively. The patients for the trial will be 400 adults (aged 18 to 75 years) who are referred and currently suffering from functional dyspepsia, as defined by the "gold standard" Rome II criteria (8). We will recruit equal numbers of males and females, and equal numbers with ulcer-like and dysmotility-like dyspepsia (see randomization below). We anticipate needing to screen approximately 800-1000 patients, to enter 500, and randomize 400. We have conservatively anticipated a drop-out of 100 patients over the entire trial, but have built in strategies to minimize dropouts.

We will carefully characterize all patients who meet the inclusion criteria before randomization, and compare the baseline measures of any patients not randomized with those included in the trial for generalizability. Patients receiving current antidepressant therapy will be excluded from the study. Subjects who have been taken off antidepressant therapy by the prescribing physician should have 30 days since their last dose of antidepressant medication.

Patients will have had in the prior 5 year, a normal esophagogastroduodenoscopy (EGD) (no esophagitis, Barrett's esophagus, cancer, erosions, or ulcer disease), and will have been diagnosed with functional dyspepsia after specialist consultation. The yield from repeating EGD in functional dyspepsia is very low. In a recent meta-analysis, it was been shown that in the absence of any "alarm" features in patients who have been diagnosed with dyspepsia, that endoscopy was not superior to empirical acid suppression. Any patients who have "alarm" features will have an EGD prior to study entry. For those without "alarm" features, a normal EGD within 5 years should be adequate (120). Patients may have failed to adequately respond to

antisecretory therapy in the past for functional dyspepsia; a good response to antisecretory therapy, which remains first line therapy, suggests underlying GERD (8,120).

H. pylori status will be tested using the *H pylori* serum test. The VIDAS HPY assay detects IgG antibodies to *H. pylori* in serum. A recent evaluation at Mayo Clinic compared the results for this test to the results for culture biopsies for 204 specimens. For this analysis, the VIDAS HPY assay showed a sensitivity of 98% and specificity of 91%. As H. pylori is highly unlikely to contribute to symptoms in functional dyspepsia (105-107), this will not be exclusion, but infection status will be considered in the analyses as a possible confounder. The results will only be disclosed at the end of the study participation to patients and the data coordinating center. Patients will be notified of positive results at the end of participation in the study by study personnel.

Symptom status will be comprehensively assessed at baseline by questionnaires in order to be able to characterize patients thoroughly. In addition, patients will be subcategorized into symptom subgroups, as recommended by the Rome II criteria based on a semi-structured interview (8):

1. Ulcer-like – predominant epigastric pain.
2. Dysmotility-like – predominant non-painful symptom: early satiety, epigastric fullness, epigastric bloating, or nausea. Symptom subgroup will be considered in the analyses.

Subjects will be required to have ceased all other drugs that could affect gastric or bowel function before enrollment into the study.

Exclusion criteria

- Any documented history of endoscopic esophagitis, or predominant heartburn or acid regurgitation, or these symptoms two or more times per week in the prior year, to exclude GERD.
- Those who have had an adequate response to antisecretory therapy according to the physician interview, to exclude patients with disease easy to control with first line therapy or misdiagnosed GERD.
- Any documented peptic ulcer disease.
- Regular use of non-steroidal anti-inflammatory drugs (except long term low dose aspirin $\leq$ 325 mg / day).
- Subjects undergoing psychiatric treatment, having a current history of drug or alcohol abuse, or currently taking psychotropic medication for depression or psychosis, or eating disorders.
- A history of abdominal surgery except appendectomy, cholecystectomy or hysterectomy, tubal ligations, bladder slings, and vasectomies.
- Subjects with concurrent major physical illness (including cardiac or liver disease, diabetes, inflammatory bowel disease, glaucoma, urinary retention, active thyroid disease, vasculitis, or lactose intolerance explaining symptoms).
- Subjects whose literacy skills are insufficient to complete self report questionnaires.
- Pregnancy or refusal to apply adequate contraceptive measures during the trial.
- Subjects currently on antidepressant therapy will be excluded.
- Patients who score 11 or greater on the 7 questions related to depression of the Hospital Anxiety Depression Scale will be excluded. These patients will be encouraged to get follow up for depression as per the IRB guidelines.
- All eligible patients over age 50 will have an EKG before randomization. Those found to have significant arrhythmias, conduction defects or a previous myocardial infarction on EKG will be excluded. Anyone with QT prolongation will be excluded.

The following concomitant medications will be prohibited during the trial:

- Systemically acting cholinergics and anticholinergics (atropine, dlidinium bromide, propantheline)

- Prokinetics (e.g. metoclopramide, tegaserod)
- Macrolide antibiotics (e.g. erythromycin, azithromycin)
- Aspirin (> 325 mg/day)
- Spasmolytics(e.g. dicyclomine)
- Antidepressants other than study medications.

Participants will be instructed to avoid grapefruit/grapefruit juice during the trial. Grapefruit/ grapefruit juice may increase the plasma concentrations of amitriptyline through inhibition of CYP450 3A4 enzymes (121).

H2 receptor antagonists and proton pump inhibitors will be allowed if patients are on a stable dose and dyspepsia persists, and they do not clinically have reflux disease, as recommended by the Rome II criteria (120).

Study coordinators will review with each patient a full list of serotonin enhancing drugs which should be avoided during the time the participant is in the study. Examples of these include monamine oxidase inhibitors, anticonvulsants, dextromethorphan (121).

IBS will not be an exclusion criterion but all patients will be required to have predominant dyspepsia (as identified on the semi-structured interview below); IBS overlaps with functional dyspepsia in at least 30% of cases (7,8,120). IBS status (by Rome II criteria) will be considered in the analyses.

Recruitment and randomization

A consecutive sample of patients will be invited to participate in the study. After informed consent has been obtained the subjects will be required to undertake at least a two-week baseline observation period. In order to ensure balance on a number of important covariates (e.g., gender, dyspepsia subtype, and psychiatric status), a dynamic allocation randomization method will be used. Treatment assignment is determined directly as a result of the distribution of assignments given the prior patients. The treatment assignation for any patient is to the treatment group with the smallest number of patients having that unique combination of stratification factors. This method guarantees that the total number on each treatment is almost always balanced when the number of patients on study is a multiple of the number of treatments involved. This approach was described by Therneau (122), based on work of Pocock and Simon (123). The general rule is that the number of categories of stratification factor combinations cannot exceed one half of the treatment group sample size (i.e. n/2). The dynamic allocation procedure works by ensuring that, as accrual proceeds, no imbalance occurs along the marginal distributions of the stratification factors across treatment arms. Allocation indications are given by the relative frequency in each categorical combination for a given stratification factor. The first patient and any situation where a tied situation exists will result in simple random allocation. Strictly speaking, a dynamic randomization procedure should be matched with permutation tests when analyzing the data. However, ample research work, including simulation studies, has shown that what type of test is used does not make a material difference. It has also been shown that the treatment allocation method has little, if any, effect on the size and power of the tests used to analyze data (124). Qualified subjects who meet entry criteria at the completion of the 2-week baseline period will be assigned to one of three treatments (tricyclic, SSRI, or placebo) using the dynamic allocation randomization described above. This procedure will aim to provide treatment group balance on several important covariates: gender, psychiatric disease (normal vs. anxiety on the HADS), dyspepsia subtype (ulcer-like vs. dysmotility-like), gastric emptying (delayed vs. non-delayed), volume inducing satiety on the nutrient drink test (low vs. normal), BMI (non-obese vs. obese), and race (Caucasian vs. non-Caucasian); stratified by Center (7 sites). Concealed allocation will be assured by use of a central web based system developed and used in past multi-center trials conducted at Mayo.

Recruitment strategies will include liaisons with other local sites for referrals (e.g. the southeastern Minnesota Mayo Clinic practice system), increased lectures to local physicians by all investigators, use of newsletters and memos, exposure on local radio/TV, representation to local support groups etc. We will undertake careful tracking systems to ensure maximum recruitment is being achieved and identify problems to fix early. Patients will be paid $200 on completing all the physiological testing ($50 baseline, $150 at end of therapy) and a further $100 for those in the accommodation sub-study ($30 at baseline, $70 at end of therapy), plus $75 for completing the questionnaires ($25 at the end of active therapy; $50 at 6 months follow-up off therapy), and reimbursement for parking and travel costs at each monthly visit. Subjects at Northwestern University Medical Center and Dartmouth-Hitchcock Medical Center will receive additional $100.00 so that the study is comparable to others at those sites and adjusted for the cost of living.

Participants that enter the study will be given a water bottle. Laminated cards will be given to physicians at each site with the diagnostic and entry criteria summarized. The number of patients to be recruited per site is realistic; no other treatment trials in functional dyspepsia are ongoing to interfere with patient recruitment. Several retention strategies have been developed. A protocol to handle possible side effects from the study medications has been developed. If side effects are addressed promptly and the participant is encouraged to continue the study, we hope to reduce dropouts related to study drug side effect. Other retention strategies include providing weekly contact, establishing a personal relationship, providing immediate and easy access to the physician, providing health information, and providing best care possible for them. Quarterly newsletters giving current health information on dyspepsia and non-pharmacological healthy living tips for dealing with dyspepsia will be provided to participants who have completed their participation until the study is complete. Annual and a study end appreciation events will be given for participants who have completed the study. The appreciation events will focus on the importance of study appreciation in improving the knowledge regarding dyspepsia, provide an update regarding dyspepsia and thank the participant.

Baseline washout

All subjects will have a two- to-four week baseline assessment period. A standardized and validated symptom diary will be completed during this baseline period (53,125). Subjects need symptoms on average of at least 2 days or more in the 2 weeks prior to randomization. Subjects will be randomly assigned to a treatment group at the end of the baseline washout period. The patients will be required to have at least moderate epigastric pain or discomfort or nausea or hunger pain on the validated GSRS (a score of $\geq$ 3 on a 7 graded Likert scale), in order to ensure change over time will be detectable (avoid a floor effect) (93,126). No placebo run-in is included as this strategy may increase the inclusion of atypical, resistant patients and is not recommended by methodological experts in the field (11,104). Four days out of a two week period of at $\geq$3 on at least one of symptoms of epigastric pain, discomfort, nausea, upper abdominal bloating / distension, early fullness after meals, or inability to finish a normal size meal.

Treatment groups

Subjects (n=400) will be randomized to one of three treatment groups for 12 weeks – tricyclic, SSRI or placebo (double dummy design). A blister pack and single nightly dosing will aid compliance.

1. Tricyclic: Amitriptyline capsule (50mg) will be taken (plus a placebo escitalopram tablet), both at night half an hour before bed. To maximize patient tolerability, in the first 2 weeks the dose of amitriptyline will be 25mg and then the dose will be increased to 50mg, but the 25mg and 50mg capsules will be indistinguishable to maintain blinding. The research pharmacy will be responsible for quality control of dosing.

2. SSRI: This group will receive the SSRI tablet escitalopram 10mg at night (plus a placebo amitriptyline capsule), both half an hour before bed.

3. Placebo: This group will take placebo escitalopram and placebo amitriptyline at night in an identical fashion. The placebos will be manufactured to ensure all tablets and capsules will be indistinguishable, and provided at the pharmacy in blister packs.

Patients will initially meet with the study coordinator for 45 minutes to explain the study in detail, establish a positive relationship, and discuss the importance of compliance and possible side effects. All study coordinators will undergo a formal training day together to standardize their performance. All patients will be given an information booklet on functional dyspepsia which provides basic information about the disorder. This information session will be standardized for all participants coming into the study. All subjects will also undergo standard medical care at each visit by a gastroenterologist (but will not receive other drugs). The gastroenterologist will encourage patients to stay in the trial despite transient side effects, and will monitor safety. The gastroenterologist will not undertake an assessment of outcomes.

Blood levels

Serum levels (trough) of amitriptyline (by HPLC) and escitalopram (by mass spectrometry) will be obtained at week 4 to confirm compliance and monitor safety, and measured in Rochester; Dr T. Moyer, PhD, Clinical Chemistry, Mayo Medical Laboratories, will consult with us to interpret the data. Blood results will be independently reviewed and investigators will be kept blinded. In the rare instance of known toxic (Amitriptyline/Nortriptyline total concentration >1000ng/ml) or very high levels (escitalopram, as judged by Dr. Moyer or designee), the result will be immediately flagged, a member of the research team will be notified by telephone there is a high level. The Safety Officer will be responsible for decisions regarding the continuation in the study of a participant with toxic drug levels. If needed, the patient will be notified to discontinue the drug, and a gastroenterologist review will occur.

Blinding Precautions

Application of a double dummy design will ensure patients, investigators and all study personnel are blinded. The side effect profile may lead to the potential problem of un-blinding despite the use of identical capsules. Notably similar side effects can occur with either drug. In order to ensure an objective, unbiased assessment of outcome the following strategies will be employed: 1) An independent assessor, blind to the treatment group will conduct all the relevant outcome assessments specified below, and 2) Valid self-report scales will be used. Patients will be specifically instructed about the importance of blinding and not mentioning side effects to the assessor gathering outcome data.

Dropouts

Every effort will be made to minimize dropouts by building in physician based support for patients. Participants will be compensated for time in the study. In the consent, we will include a proviso that subjects who dropout can elect to continue with the follow-up (plus remuneration) or if this is not agreed, then we will request their permission to review primary outcome data from the medical records and the patients' physician. If this fails, the primary unobserved outcomes will be imputed. Specifically for the primary endpoint (adequate relief for any 5 of the last 10 weeks of active treatment), subjects who do not provide a response on any particular week will have that week considered as "No relief". Subjects with less than 10 weeks of reported data may thus still qualify as "responders" if they have at least 5 of the last 10 weeks with reported adequate relief. Continuous endpoints (e.g. QOL measures and satiety test responses) will be imputed for those with missing values. The method of imputation will depend on the proportion of missing values and the frequency the measures are made (e.g. monthly versus only at the completion of the 12 week treatment period). Thus for example, in the analysis of the nutrient drink test at week 12, the missing data will be imputed using the overall subjects (with non-missing data) mean value. A corresponding adjustment to the error degrees of freedom in the ANCOVA models will be made to accommodate this imputation (i.e. subtracting one degree of freedom for

each value imputed). Although this approach provides an unbiased estimates of treatment effects under the null hypothesis it can be overly conservative if the missing data rate is more than 20-25%, in which case other multiple imputation methods will be considered (i.e. a regression method using baseline pretreatment as the regressor variable to impute the missing post-treatment values and a bootstrap approach to obtain the appropriate estimate of residual variance). For monthly continuous measures, a "last rank carried forward" method will be considered. We will also consider including an analysis of time to dropout using survival analysis methods to identify potential predictors associated with dropout rate which can provide information on the missing at random assumption. We will record reasons for dropout as they occur and offer subjects dropping out the option of completing all assessments required at the last visit including physiological testing. Adverse event forms will be completed if relevant.

Follow-up (6 months)

Unscheduled use of antidepressants will be prohibited during the follow-up phase off therapy unless a medical need arises (e.g. major depression), as this would confound evaluation of outcomes. Physicians looking after patients will be informed in writing. Any medication use or intervention and the reason will be recorded. Use of anti-dyspepsia medications will be analyzed as a secondary outcome measure of treatment failure.

Measures

The subjects will complete a set of measures to assess global improvement, symptom severity, quality of life, anxiety, depression, physiological measures and genotyping, as summarized below. We have extensive experience with all of these assessments, and respondent burden will not be an issue in this trial based on our experience

<u>Symptoms and diagnostic evaluation</u>

a. Bowel Disease Questionnaire. To ensure that a reliable diagnosis will be made according to the Rome II criteria, the validated self-report bowel disease questionnaire will characterize patients' symptoms of functional dyspepsia in detail at baseline. It is very widely used, and was developed and validated by us (92); it now includes the Rome II diagnostic criteria. Six questions will be used to screen patients for functional dyspepsia. These questions were taken from the Rome III validation study and have acceptable face and content validity (Whitehead, Talley et al, in preparation).

b. Semi-structured interview. To assess symptom subgroup, a semi-structured interview will be undertaken by the study coordinator at baseline, the end of therapy and the end of follow-up to determine the predominant dyspepsia symptom. We expect most patients to report multiple dyspepsia symptoms, but assessment of the major (predominant) symptom has been shown by us to be a useful method for subdividing patients according to the Rome II criteria and predicting response to therapy (8,53). In addition to identification of symptoms, on baseline the participant will be asked to identify when they believe symptoms started and whether they had known exposure to selected infectious agents. No self-report measures are established to provide a valid means of subdividing functional dyspepsia, and hence the interview. The baseline assessment will be included in models testing the predictive value of dyspepsia subgroups. The stability of the predominant symptom on follow-up will also be tested by reassessment at the end of therapy and the end of follow-up. In addition to the detailed symptom information, information will be collected on allergies, history of asthma or seasonal allergies, and food intolerances. The interviewer will do this before any other assessments, and will be blinded to other outcome measures when doing the interview.

c. Individual dyspepsia symptoms. A validated diary card will be completed daily over the 12 weeks on therapy and prior to the last study visit: it will measure epigastric pain, epigastric discomfort, early satiety, bloating, fullness and nausea on 4 graded scales (0, nil; 1 mild; 2 moderate; 3 severe) (53,109). The diary is

established to be responsive to change, and has been used successfully in previous large trials. A well-validated more comprehensive self-report measure, the Gastrointestinal Symptoms Rating Scale (GSRS) (employing 7 graded Likert scales) will assess all dyspepsia and IBS symptoms over the prior week, and will be completed at every monthly visit on therapy and at the last follow-up visit; it is responsive to change (93,125).

d. Treatment compliance. Compliance will be measured by tablet count at each monthly visit on therapy, and a checklist will be completed by the study coordinator. Participants will complete a medication diary while in the treatment phase.

e. Health status. Number of other physician visits, new investigations and any other medications being taken will be recorded at each visit.

f. Side effects. These will be recorded at each visit by a gastroenterologist not involved in the assessment of outcomes.

Global evaluation

g. Adequate relief of dyspepsia symptoms in the prior week (primary outcome). This simple self-report global measure is widely accepted and has been tested for responsiveness in functional dyspepsia (12,128). We will ask patients to answer the following yes/no question once a week: "In the past 7 days, have you had adequate relief of your stomach symptoms?" It will be evaluated weekly as part of the self-report diary over the 12 weeks on therapy, and monthly by phone over the 6 months of follow-up after therapy. A responder will be defined as a study patient who answers "yes" for at least 50% or greater of the weeks of treatment 3-12 (10 weeks). The first two weeks of treatment will not be included to allow for steady state to be established (12,127). The adequate relief measure is considered a clinically relevant and robust endpoint (128-130), and this type of measure is now very widely accepted by consensus panels in IBS trials as the primary outcome (104).

h. Clinical Global Impressions (CGI). This is a widely used, responsive and well-accepted global measure of treatment improvement or deterioration in antidepressant trials. The CGI will be administered by a trained study coordinator (127), and will be assessed at baseline, and every monthly visit.

Psychological Measures and Psychiatric Diagnoses

i. The Symptom Check List 90 (SCL-90) is a measure of psychological state that will be done at baseline, end of treatment phase and end of study. Questions use a 5-point Likert scale. There are 9 scales; somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism (131).

j. The Somatic Symptom Checklist (SSC) will be done at baseline, end of the treatment phase and at the end of the study. The SSC measures the frequency and bothersomeness of 12 non-gastrointestinal symptoms on a five-point Likert scale. The overall mean score can be computed for each subject; (132).

k. Hospital Anxiety Depression Scale (HAD). HAD is a self-report 14 item scale, which is recommended for detecting mild mood disorders in non-psychiatric outpatients, and has been used with patients with IBS (133,134). This measure does not include physical symptoms thus avoiding confounding across groups; measurements will occur at baseline, end of 12 weeks on therapy, and at the end of the follow-up.

l. Profile of Mood States (POMS) will be done at screening, end of treatment phase and end of study. The POMS is a 65 item survey with six subscales (135).

m. The State Trait Anxiety Inventory (STAI) is a self report inventory of baseline and situational anxiety measuring both positive and negative emotional states (136). The STAI will be administered at screening, end of treatment phase, and at the end of the study.

n. The Pittsburgh Sleep Quality Index (PSQI) will also be done at baseline, at end of treatment phase, and end of study. The PSQI contains 19 self-rated questions with 7 "component" scores (137)

o. The Eating Disorder Examination-Questionnaire will be administered at baseline, and at the end of treatment and the end of the study to assess for undiagnosed binge eating. This is a self-administered questionnaire that has demonstrated good identification and differentiation between infrequent binge eaters and recurrent bingers.(149, 150)

p. The Early Trauma Inventory Self Report – Short Form (ETISR-SF) is a 29 item scale that has been reported to be a valid and reliable measure of childhood trauma (149). The ETISR-SF will be administered at the last visit. In addition, the Drossman abuse questionnaire for children and adults will be administered at the last (exit) visit. Functional bowel symptoms and a prior history of sexual or physical abuse as a child or adult have been found to be associated (151, 152, 153).

Severity Index

q. Functional Bowel Disorder Severity Index (FBDSI) (138). A valid and reliable index, FBDSI = pain intensity (0-100) + 106 X (diagnosis of chronic functional abdominal pain (1= yes, 0= no) + 11 X (# physician visits / 6 months). It quantifies disorder severity over the last 6 months, it will be used to define mild (scores <35), moderate (scores 36-109) and severe functional dyspepsia cases (scores >109). The FBDSI is highly correlated with both degree of functional disability and a physician's independent ratings of illness severity (138). This will be measured at baseline and the end of the 6 month follow-up phase off treatment.

Quality of life

r. Generic: Medical Outcomes Study Short Form 36 (SF36) (139). The SF-36 is a 36 item self-administered questionnaire, based on a multi-dimensional model of health. Eight subscale scores may be derived from the 36 items: physical functioning, physical role limitation, emotional role limitation, bodily pain, mental health, social functioning, vitality and general health perceptions. The instrument has been used in a wide variety of settings and extensive normative data are available; it is responsive in functional GI disorders (140).

s. Disease specific: Nepean Dyspepsia Index (NDI) (94,95). The NDI is a valid, responsive self-report scale developed by us that assesses the impact of functional dyspepsia on 5 dimensions of health status over the prior 2 weeks: tension/sleep, interference, eating/drinking, knowledge/control, and work/study. Both the SF-36 and NDI will be measured at baseline, then at each monthly visit on therapy.

Physiologic and genetic measurements

t. Gastric emptying

A validated, standard test (141) using the same meal and acquisition protocol at all sites will be done twice: in the second baseline week prior to randomization and at week 12 on therapy. Although of uncertain relevance, in menstruating females, the baseline will not commence until the beginning of the menstrual cycle to standardize the timing of transit and all our physiological measurements (142).

u. Assessment of Early Postprandial Sensations by Means of a Standardized Liquid Nutrient Test

All subjects will complete this test at baseline and on therapy at 12 weeks (71).

v. Measurement of Gastric Volume and Accommodation Response by SPECT

This test will be undertaken only at the Rochester site in a sub-study (n=20 in each arm of the trial) twice, at baseline and at week 12 on therapy (69,70). We expect to study most patients twice based on our experience but have calculated for possible drop-outs in the planned analyses. The randomization will be stratified across the sites, such that an equal number of patients will be randomized to each treatment arm in Rochester.

w. Genetic Analyses for Detection of Polymorphisms

Using a functional candidate gene approach, we considered 2 gene disorders to be potentially important in treatment response in functional dyspepsia as they may affect gut function and are linked to psychiatric co-morbidity: 5HTTLPR and GNβ3. We will also genotype genetic variants that may be related to TCA and SSRI pharmacogenomics which may impact response to therapy. The genes to be evaluated include: *CYP2C19, CYP2D6, CYP3A4, CYP2C9, SLC6A4,* and *HTR2A.* Due to recent findings we would like to genotype six

additional SNPs which have shown a strong correlation with FD symptoms. These SNPs include: rs2049129, rs1887589, rs6573434, rs4835546, rs9524690 and rs12436333.
Genetic analysis: Approximately 30 ml of blood will be drawn by a trained phlebotomist and collected into a standard tube with EDTA at baseline. Any blood left from other lab work will be frozen to assure adequate samples for DNA analysis provided the participant has agreed as outlined below. Fresh whole blood will be extracted using Puregene™ Reagents from the Gentra Corporation using either manual methodology or the Gentra Autopure™ automated DNA extractor. After extraction, all DNA samples will be tested by spectrophotometry using the SPECTRAmax PLUS 384 spectrophotometer from Molecular Devices. All samples will be diluted to a standard concentration of 0.25ug/uL. Molecular analysis will be performed using previously described primers specific for 5-HTTLPR (5HTT) and C825T (GNβ3) polymorphisms. The 5-HTTLPR polymorphism will be typed by polymerase chain reaction (PCR) using flanking primers (forward 5'-GGCGTTGCCGCTCTGAATGC-3' and reverse 5'-GAGGGACTGAGCTGGACAACCAC-3') (78). The C825T polymorphism will be typed using the forward primer, 5'TGACCCACTTGCCACCCGTGC-3' and 5'-GCAGCAGCCAGGGCTGGC-3' (143). PCR will be performed by the Mayo Clinic Cancer Center Microarray and Molecular Epidemiology Shared Resources core facility using Applied Biosystems (TaqMan®) technologies. All patients recruited at baseline will have DNA extracted if they agree in order to assess the generalizability of the trial participants, but patients will not be excluded if they do not wish to undertake this part (refusal rates are expected to be very low based on our previous experience).
x. Safety data
A full blood count and chemistry panel will be collected at baseline, 4 and 12 weeks for safety monitoring, and centrally processed at Mayo Medical laboratories. Pulse and blood pressure will be measured at each monthly visit. Data on adverse events and serious adverse events will be collected as is standard.

TREATMENT PROTOCOL

1. Study sites: There will be six sites -
a) Mayo Clinic, Rochester, MN: recognized as a major medical center for management of functional GI disorders, under the direction of Dr. Talley who is nationally and internationally recognized for his research into functional dyspepsia, epidemiology, clinical trial design and multicenter trials. Dr. Camilleri is a consultant with extensive experience in all the physiological and pharmacogenetic studies planned, has developed and validated many of the techniques and will oversee these tests in the trial. Dr. Locke (consultant) has extensive experience with outcome evaluations in the functional GI disorders including functional dyspepsia. Dr. Zinsmeister is a very experienced statistician and his team will coordinate the data management and analysis efforts. Dr. Mrazek (consultant) is a psychiatrist with a special interest in the genetics of psychiatric disease and will assist with data interpretation.
b) Mayo Clinic, Scottsdale, Arizona: Dr. Di Baise has a major research interest in functional bowel disorders and will co-ordinate this site. The practice is large and sees a diverse range of functional GI patients, and the tests proposed are already set up.
c) Mayo Clinic, Jacksonville, Florida: Dr. Bouras trained with Dr. Camilleri in research methods in GI motility, and Dr. Achem is an experienced clinical GI investigator with a motility interest; the practice is large and sees a diverse range of functional GI patients, and the tests proposed are already set up.
d) Northwestern Memorial Hospital, Chicago: Dr. Jones is an experienced clinical investigator, and the GI practice is large and diverse; he is very experienced with GI motility testing.
e) Saint Louis University School of Medicine: Dr. Prather trained with Dr. Camilleri, is an experienced clinical investigator, her GI practice is large and diverse, and she is an expert in and very experienced with GI motility testing.

f) Dartmouth-Hitchcock Medical Center, New Haven, New Hampshire: Dr Brian Lacy is an experienced clinical investigator in a large GI practice. He is an expert in gastrointestinal motility.
g) Baylor College of Medicine, Houston, Texas: Dr Hashem El-Serag is an experienced clinical investigator in a large GI practice and diverse population.
h) McMaster University Medical Centre, Hamilton Health Sciences. Dr. Paul Moayyedi is highly experienced in clinical research. He is a Professor of Medicine and Director of Gastroenterology.

1. Randomized controlled trial (all sites): The acute treatment phase will comprise 12 weeks in total. Patients will be evaluated at a visit every 4 weeks for 12 weeks on therapy, plus monthly for 6 months once treatment is ceased. A daily diary card will be completed throughout the 12 week active treatment phase, and at the 6 month follow-up. A case report form is provided in Appendix 1. Five years will realistically be required to complete the study; each patient randomized will require 9 months in the trial. We anticipate each center will each month of recruitment be required to screen approximately 6-8 new patients to identify 4 potential cases, of whom 1-2 will be randomized, plus schedule and complete visits for all patients in the trial, as well as attend to data management.

2. Gastric emptying: Gastric emptying of solids will be done after an overnight fast, applying a standard meal and acquisition protocol at 0, 30 minutes, 1, 2, 4 and 6h at baseline (all recruited) and at 12 weeks on therapy. We will not exclude participants from the trial who are not willing to undergo this or other physiological tests, but expect this number to be very small (<5% non-participation for this test).

3. Assessment of Early Postprandial Sensations by Means of a Standardized Liquid Nutrient Test: High symptom scores in the satiety test are associated with impaired gastric hypersensitivity and possibly impaired accommodation. Volume of Ensure® to full satiation and symptom scores in 73 healthy adults and adolescents have been reported from our laboratory (98). The primary end points are the volume ingested and aggregate symptom score at 30 minutes. Symptom scores will be evaluated at baseline, 15 minutes and 30 minutes post ingestion and at satiety. The test will be done at baseline (all recruited) and 12 weeks on therapy.

4. Sub-study of gastric accommodation and antidepressant therapy
Inclusion criteria - All patients at the Rochester, Jacksonville, and Scottsdale sites will have been fully investigated within the previous 5 years, with studies performed to exclude organic diseases. We anticipate studying 60 patients at baseline (all recruited) and at least 45 patients at 12 weeks on therapy (assuming a conservative 25% drop out rate).

Methods
We have validated a noninvasive method to measure the gastric accommodation response that requires imaging of the gastric mucosa, use of a SPECT camera for three dimensional imaging, volume rendering and calculation of the total gastric volume.

a) Imaging of gastric mucosa - The entire gastric mucosa [both parietal (oxyntic) cells and non-parietal (mucous) cells] is able to take up and excrete $^{99m}Tc0_4^-$ from the circulating blood pool. Radiation exposure (10 mCi) is within permissible ranges for research and clinical studies.
b) SPECT imaging - Tomographic studies are acquired on a large field of view dual-head gamma camera system with subjects supine and detectors over the upper and mid abdomen. Ten minutes after i.v. 10 mCi $^{99m}TcO_4^-$, dynamic tomographic acquisition is performed with 3 complete 360° orbits at 10 minutes per orbit: 1 orbit fasting and 2 after 300 kcal Ensure® (Ross Products, Abbott Laboratories, Columbus, OH). Images are

acquired into a 128 x 128 matrix, every 6° at 3 seconds per image, and transaxial images of the stomach are reconstructed using filtered back-projection.
c) Processing for quantitation of gastric volume -Transaxial images are transferred via DICOM to a desktop Windows NT workstation. The stomach is identified using a semi-automated intensity-based extraction algorithm (Object Extractor, Analyze^{AVW} PC 2.5, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN), which has been used previously in volumetric imaging studies. Total gastric volume is measured fasting (for 10 minutes) and during two ten-minutes postprandially. The average volume is calculated.
d) Experimental protocol: SPECT accommodation study - This test will be performed at 7:00 a.m. after an overnight fast at baseline and again at week 12. Endpoints for analysis will be the changes in gastric accommodation response, the volume to full satiety and aggregate symptom score with the satiating meal.

All physiological testing will be undertaken at 7am after an overnight fast to control for circadian/diurnal cycles and to reflect clinical practice. We will assess gastric function at baseline, and on treatment after taking the last dose of amitriptyline, escitalopram or placebo the evening before at 10pm. As testing will be done during steady state and the drugs have a long half-life, this design is optimal in terms of clinical interpretability.

Evaluation and visit schedule										
	Visit:	**1**	**2**	**3**	**4**	**5**	**6**[4]	**7**	**8-11**	**12**
Evaluation	**Day:**	**-14**	**-7**	**1**	**28**	**56**	**84**	**112**	**140**	**252**
Evaluation	**Month**	**0**	**0**	**0**	**1**	**2**	**3**	**4**	**5-8**	**9**
Informed consent		X								
Inclusion/exclusion criteria		X								
Demography/background information		X								
Past/current medical/surgical conditions		X								
Functional dyspepsia history		X								
Physical examination		X					X			
Vital signs		X	X	X	X	X	X			X
EKG		X								
Blood count and serum chemistry		X			X		X			
Blood for polymorphisms		X								
H. pylori test		X								
Urine pregnancy test		X[1]					X[1]			
Blood levels for amitriptyline and escitalipram					X					
Dispense study medication				X	X	X				
Collect diaries[2]			X	X	X	X	X			
Collect study medication/compliance					X	X	X			
Bowel Disease Questionnaire 6 (BDQ-6)		X								X
Functional Bowel Disease Symptom Index (FBDSI)		X								X
GSRS		X		X	X	X	X			X
Health Status (physician visits, other medications)				X	X	X	X	X	X	X
Adverse Events (Weekly and as notified)					X	X	X	X	X	X
Global Evaluation-Adequate relief (Weekly)				X	X	X	X	X	X	X
Clinical Global Impression (CGI)				X	X	X	X	X	X	X

Semi – Structured Interview		X					X			X
Evaluation and visit schedule										
	Visit:	**1**	**2**	**3**	**4**	**5**	**6[4]**	**7**	**8-11**	**12**
Evaluation	**Day:**	**-14**	**-7**	**1**	**28**	**56**	**84**	**112**	**140**	**252**
Evaluation	**Month**	**0**	**0**	**0**	**1**	**2**	**3**	**4**	**5-8**	**9**
Pittsburgh Sleep Quality Index		X					X			X
Symptom Check List 90 (SCL-90)		X					X			X
State Trait Anxiety Inventory (STAI)		X					X			X
Profile of Mood States (POMS)		X					X			X
Somatic Symptom Checklist (SSC)		X					X			X
EDE-Q		X					X			X
ETISR-SF										X
Adult Trauma questions from BDQ 4)										X
HADS		X					X			X
SF-36		X		X	X	X	X			
NDI		X		X	X	X	X			
Gastric Emptying			X				X			
Liquid nutrient test			X				X			
Gastric accommodation sub study (SPECT)			X [3]				X[3]			

[1] Women of childbearing potential. [2] To maximize diary compliance, study coordinators will call patients weekly with a reminder to update the daily diary. [3] Will be conducted at Mayo Clinic Rochester only. [4] Participants in Gastric accommodation sub study (SPECT) may have additional two weeks due to scheduling of tests.

Statistical Analysis and Power

Note – an interim analysis has been requested by the Data Safety Monitoring Board and can be found in Appendix A

Specific Aim 1:

i) Assess whether antidepressant therapy is more efficacious than placebo in relief of the symptoms of functional dyspepsia, adjusting for psychological and psychiatric co-morbidity. We will also examine whether antidepressant therapy reduces disability and improves quality of life in functional dyspepsia. All subjects randomized will be included in the primary analysis based on the intent-to-treat principle. We anticipate a 20-25% dropout rate, and as noted on page 19, subjects with missing reports of adequate relief on any particular week will have that week considered as "No relief". Thus subjects with more than 5 weeks of missing 'adequate

relief' indicators would be counted as treatment failures; this has been considered in the power calculations. In order to assess external validity, relevant clinical data in eligible patients will be analyzed to assess both their association with participation, and baseline data in the three arms will be examined for their association with completion of treatment (Yes/No) using logistic regression.

Primary endpoint: This will be adequate symptom relief (Yes/No) in any 5 of the last 10 weeks of active treatment by intention-to-treat. This endpoint has been standard in recent functional dyspepsia trials (12,128) and many recent IBS trials (45,129,130); we will also compute the average number of weeks with adequate relief in the first and last month (each using a denominator of 4 weeks) as well as over the entire 12 weeks for each patient as secondary outcomes. Adequate relief will be compared overall among the treatment groups incorporating the7 sites as strata using the Cochran-Mantel-Haensel (CMH) test statistic for general association (on 2 degrees of freedom). Individual site assessments of the direction of treatment effects will be informally examined for homogeneity by computing site-specific odds ratios (and 95% CIs) for symptom relief in each active treatment relative to the placebo group. In addition, a logistic regression model with global symptom relief (based on the last month of treatment as described above) as the binary dependent variable will be used to test for an increased odds for relief in the active treatment groups (each relative to the placebo reference group) adjusting for the covariates used in the dynamic allocation randomization. It is commonly recommended that stratification variables in a randomized trial setting be included in the analysis of treatment effects, particularly important prognostic covariates. Although its long been known that this is clearly important in linear models due to the reduction in the residual error variance, it has also been recommended for the logistic regression model setting (144). The primary analysis was specified as the CMH test with the six sites as a stratification factor to compare overall symptom relief percentages among the three treatment groups. It is very often recommended that site be incorporated in the analysis of multicenter studies. Specific two factor interactions of each active treatment group (dummy variable coding) with each of the primary (binary coded) covariates will also be examined in a further model. The overall test for 2-factor interactions (with treatment group) will be based on the difference in log-likelihood values between the main effects model (no interactions) and the model with all 2-factor interactions of covariates with treatment group, except for treatment center. If the homogeneity of treatments effects across centers appears untenable based on the informal assessment noted above, this latter analysis examining 2-factor interactions will focus on an exploratory analysis of which covariates may have produced differential treatment impacts at the different centers, as opposed to formal assessment of treatment effect modification. Per protocol analyses will also be considered in the secondary analyses.

We will also explore whether an antidepressant is more efficacious than placebo in relief of symptoms in functional dyspepsia symptom subgroups. The subset of subjects randomized to active treatment (tricyclic or SSRI) will be used to assess the odds for global symptom relief in those randomized to tricyclic anti-depressant relative to those assigned to SSRI. These odds ratios (and 95% CIs) will be computed separately for ulcer-like dyspepsia and dysmotility-like dyspepsia subgroups via the appropriate dummy variable coding of the four combinations (treatment group by dyspepsia subgroup). Using the corresponding estimated coefficients from a logistic regression model adjusting for other potentially important covariates (e.g., gender and IBS diagnosis), a formal test for equal odds ratios in the two dyspepsia subgroups will be based on the corresponding linear contrast of the estimated coefficients. Similar analyses focusing on the subgroups with vs. without early satiety on the nutrient drink test, and separately, delayed vs. normal gastric emptying will also be examined. Although any of the four subgroups (active treatment by dyspepsia subgroup) could be used as the reference group in the analysis for Aim 1, the ulcer-like dyspepsia subgroup on placebo would seem to be a logical choice. This will be a secondary analysis; the primary analysis was described in the previous paragraph.

Dropouts

Every effort will be made to minimize dropouts by building in physician based support for patients. Participants will be compensated for time in the study. In the consent, we will include a proviso that subjects who dropout can elect to continue with the follow-up (plus remuneration) or if this is not agreed, then we will request their permission to review

primary outcome data from the medical records and the patients' physician. If this fails, the primary unobserved outcomes will be imputed. Specifically for the primary endpoint (adequate relief for any 5 of the last 10 weeks of active treatment), subjects who do not provide a response on any particular week will have that week considered as "No relief". Subjects with less than 10 weeks of reported data may thus still qualify as "responders" if they have at least 5 of the last 10 weeks with reported adequate relief. Continuous endpoints (e.g. QOL measures and satiety test responses) will be imputed for those with missing values. The method of imputation will depend on the proportion of missing values and the frequency the measures are made (e.g. monthly versus only at the completion of the 12 week treatment period). Thus for example, in the analysis of the nutrient drink test at week 12, the missing data will be imputed using the overall subjects (with non-missing data) mean value. A corresponding adjustment to the error degrees of freedom in the ANCOVA models will be made to accommodate this imputation (i.e. subtracting one degree of freedom for each value imputed). Although this approach provides an unbiased estimates of treatment effects under the null hypothesis it can be overly conservative if the missing data rate is more than 20-25%, in which case other multiple imputation methods will be considered (i.e. a regression method using baseline pretreatment as the regressor variable to impute the missing post-treatment values and a bootstrap approach to obtain the appropriate estimate of residual variance). For monthly continuous measures, a "last rank carried forward" method will be considered. We will also consider including an analysis of time to dropout using survival analysis methods to identify potential predictors associated with dropout rate which can provide information on the missing at random assumption. We will record reasons for dropout as they occur and offer subjects dropping out the option of completing all assessments required at the last visit including physiological testing. Adverse event forms will be completed if relevant.

Sample size

Primary aim: Assuming that treatment effects are homogeneous across study centers, an overall pooled comparison of each active treatment vs. placebo would require the number of subjects per treatment group listed in the table below to achieve 80% power at a two-sided alpha level of 0.025 (i.e., adjusting for two pairwise tests, each active drug against placebo). We consider a 20% therapeutic gain or greater over placebo to be clinically significant. The logistic regression model analysis incorporating potentially important covariates (gender, psychiatric status-HAD anxiety scale $\geq$11, dyspepsia symptom subtype, gastric emptying, satiety with $\leq$800 ml. being abnormal (71), and race) should provide similar or better power to detect comparable treatment group effects assuming no substantial interactions with covariates or differential site effects. We assume a 25% drop out rate in each arm. Therefore, the planned recruitment of 133-134 patients per arm (total 400 patients) will provide sufficient power in the ITT analyses.

Placebo response rate	Active treatment response rate	N per group required*	Placebo response rate	Active treatment response rate	N per group required*
20%	40%	98	35%	55%	116
	45%	65		60%	75
	50%	46		65%	52
25%	45%	107	40%	60%	118
	50%	70		65%	75
	55%	49		70%	51
30%	50%	113	45%	65%	116
	55%	73		70%	73
	60%	51		75%	49

Assigning the 'dropouts' (those with more than 5 weeks of unreported responses) as treatment failures could attenuate the treatment differences, but an ITT analysis must include all subjects randomized and it seems

the dropouts would more likely be treatment failures in this setting. Since the dropouts would be unlikely to report symptom relief, little misclassification bias should result. Thus, a 'true' treatment difference of 25% on placebo and 45% on active drug should be reflected in the observed symptom relief proportions. It is difficult to specify a minimally clinically relevant difference, but certainly differences of 20-25% or greater would be of interest, while smaller size differences would be less important. Although the inclusion of important covariates (particularly the stratification factors) may inflate the standard errors of the logistic regression coefficients, the coefficients themselves would likely also increase in absolute value (144). Since the stratification factors will be uncorrelated with treatment assignment by design, this should improve the overall efficiency of the analyses.

An exploratory analysis will assess symptom subgroups and treatment outcome. It is anticipated that the functional dyspepsia subgroups (ulcer-like vs. dysmotility-like dyspepsia) will have a roughly even split (50% vs. 50%) over all centers (e.g. yielding 50 to 70 per subgroup overall). The test for equal treatment effects in both subgroups is equivalent to testing the equality of the proportions with global symptom relief between two combined categories: ulcer-like (tricyclic) and dysmotility-like (SSRI) vs. ulcer-like (SSRI) and dysmotility-like (tricyclic). The table below lists the power to detect a difference in these two proportions for several anticipated treatment response rates in each subgroup (2-sided alpha level of 0.05 i.e. for a single test of differential subgroup effects).

N per subgroup	% with Symptom Relief				Power (%)
	U-L(T)	D-L(SSRI)	U-L(SSRI)	D-L(T)	
50	25	25	40	40	62%
50	25	25	45	45	85%
50	30	30	50	50	83%
50	30	30	55	55	95%
60	25	25	40	40	70%
60	25	25	45	45	91%
60	30	30	50	50	89%
60	30	30	55	55	98%
70	25	25	40	40	77%
70	25	25	45	45	94%
70	30	30	50	50	93%
70	30	30	55	55	99%

The proposed analyses for potential interactions among subgroups and treatment assignment are exploratory analyses aimed at identifying potentially more responsive subsets of patients. It's conceivable that the differences in response rates (placebo vs. active treatment) may be much larger in some subgroups (e.g. 30% vs. 60% in dysmotility-like FD) in contrast to other subgroups (e.g. 20% vs. 30% in ulcer-like FD). Assuming an equal number of both types of patients in each treatment arm would then reflect an overall difference in response rates of 20% (e.g. 25% vs. 45%) for placebo vs. active drug. It is only these rather large "differential" response rates (interactions) that would be clinically relevant to detect.

ii) Symptom Relapse : whether after cessation of therapy in responders, the clinical benefit persists longer following an antidepressant than placebo over 6 months of follow-up.
During the 6-month observational follow-up period post treatment, subjects are followed monthly. Based on the interviews, a date of ‘symptom relapse’ (see definition above of adequate relief) will be ascertained. The date

of last follow-up at 6 months if no relapse occurs will be considered a censoring date (or last contact date if the subject is lost to follow-up before 6 months). A proportional hazards regression analysis in only those subjects with global symptom relief at the end of the 12-week active treatment phase will be included in this analysis. The primary focus of this analysis is to identify potential covariates associated with time to relapse and estimate the 'hazard ratios' (with 95% CIs) for each active treatment (relative to placebo) used in the 12-week treatment phase.

Sample size

Assuming roughly 40% of the 400 total subjects over all three treatment groups achieve global symptom relief at the completion of the 12-week active treatment phase, there should be approximately 160 subjects to assess the association of specific covariates with 'survival free of relapse' (roughly 133 who were on antidepressant therapy and 27 on placebo). If the event rate (relapse) in subjects on active treatment was 20% and was 80% for subjects on placebo, there would be roughly 48 events available to assess the association of potential covariates with relapse free survival. Assuming an exponential survival free of relapse, there is better than 90% power (e.g., using the log rank test at $\alpha = 0.05$) to detect a difference in the proportions without relapse (combined active treatment groups vs. placebo) of .8 versus .35, or .7 versus .25.

Specific Aim 2:

i) Assess whether gastric emptying (motor dysfunction) and the nutrient drink test (a test of gastric hypersensitivity and/or gastric accommodation) is altered by antidepressant therapy with a tricyclic or SSRI, and whether variation in physiologic responses are associated with treatment outcome. The effect of treatment on gastric emptying will be assessed using an analysis of covariance incorporating gender, site, and the baseline gastric emptying summary as covariates. The anticipated endpoint to be used will be the proportion ($prop_{2\ hr}$) of meal emptied from the stomach at 2 hours (first transforming to $\sin^{-1}(\sqrt{\ })$ scale). Pairwise comparisons of each active treatment group vs. placebo will be examined assuming the absence of substantial site or gender by treatment interactions. A similar analysis of the (log) max-tolerated volume and the aggregate symptom score in each subject will be examined to assess treatment effects on satiety as measured using the nutrient drink test. The association of post-treatment gastric emptying with global symptom relief will be based on a multiple linear regression model with the gastric emptying summary $\left[\sin^{-1}\left(\sqrt{prop_{2hr}}\right)\right]$ as the dependent variable and symptom relief as the primary predictor variable, with other covariates included as potential confounders (e.g., gender, site, and dyspepsia subgroup). Similarly, the association of (log) max-tolerated volumes and aggregate symptom scores with global symptom relief will be assessed using multiple linear regression.

ii) In a sub-study, directly test whether impaired gastric accommodation (by SPECT) is altered by an SSRI or tricyclic antidepressant. In the subset of subjects who have gastric accommodation measured, the primary analysis will focus on gastric accommodation (actual difference between postprandial and fasting volumes) and secondarily the individual fasting and postprandial volumes. We have already characterized the normal accommodation responses of healthy volunteers with ages ranging from 18-60 years (98). An analysis of covariance will be used to compare treatment groups. A logistic regression model with global symptom relief as the binary dependent variable and gastric accommodation difference as the primary predictor variable will be used to assess the association of symptom relief vs. the relative change in gastric volume following a meal. In addition to the accommodation difference measured at baseline, other potentially important covariates (e.g., gender, BMI and dyspepsia subgroup) will be considered for inclusion in the model.

The association between gastric accommodation versus the (log) max-tolerated volume, and versus the aggregate symptom score will be estimated via the Pearson product moment correlation. A formal test for equal associations among treatment groups will be examined using linear regression models with a dummy variable coding of treatment groups and specific interaction terms of these dummy variables by (log) max volume and separately aggregate symptom score.

Sample Size

The table below summarizes data for the primary response measures and assumes a relative variation (CV%) slightly bigger than in previously observed data to accommodate site-to-site variation. The coefficients of variation (CV%) are based on data using the same methods. The estimated effect size detectable with 80% power using a two sample z-test (i.e., assuming the variation values are known) at a two-sided alpha level of 0.05 is listed for a number of potential group sizes. The effect size is the difference in group means as a percentage of the overall mean for each response and assumes 80-100 subjects per group for the gastric emptying and nutrient drink test outcome measures, and 15-20 subjects per group for the gastric accommodation response variables. The Analysis of Covariance (using body mass index as a covariate) should provide 80% power to detect similar differences across all the groups.

Response	Mean (SD)	CV(%)	N per group	Effect Size[#] (%)
Solid gastric emptying (% emptied by 2 hrs)	0.58% (0.16)	29%	80 100 120	14 13 12
Solid gastric emptying (% emptied by 4 hrs)	0.96 (0.08)	8%	80 100 120	4 4 3
Maximum tolerated Vol. (Nutrient Drink Test)	1306 (373)	35%	80 100 120	17 15 14
Aggregate Symptom Score Nutrient Drink Test	166 (72)	45%	80 100 120	22 20 18
Fasting Gastric Volume (SPECT)	236 (69)	30%	15 20	34 29
Postprandial Gastric Volume (SPECT)	755 (103)	14%	15 20	16 14
Delta Gastric Volume (Post Prandial-Fasting)	519 (94)	18%	15 20	20 18

Effect size is the difference between (2) groups as a percentage of an overall (both groups) mean. Based on two-sample z-score test at α=0.025 (i.e., adjusted for two pair-wise comparisons). It should be noted that in our new pilot data, the CV% for the aggregated symptom score was 44%, and 34% for the maximum tolerated volume which are virtually identical to those used for the sample size estimates in the table based on prior studies at Mayo.

Specific Aim 3:

Examine whether polymorphisms of the heterotrimeric G protein and serotonin reuptake transporter predict outcome in functional dyspepsia patients receiving antidepressant therapy. Allele frequencies (%) for the L and S allele (for 5-HTTLPR) and C and T allele (for C825T) will be generated. Genotype frequencies (%) for LL, LS, and SS (for 5-HTTLPR) and CC, CT, and TT (for C825T) will also be generated. The association between specific polymorphisms of the G protein and serotonin reuptake transporter vs. global symptom relief in response to treatment will be assessed based on a logistic regression model. The primary focus of this aim is to estimate the odds ratios (and 95% CIs) for global symptom relief in subjects with a specific polymorphism relative to those without the polymorphism. The odds ratios (and 95% CIs) for global symptom relief in those with the specific polymorphism (relative to those without) will be estimated using the coefficients from the logistic model to predict symptom relief. These will be computed separately for each treatment group and graphically displayed; a similar graphical assessment for other subgroups will also be examined.

In addition, the association between specific physiologic responses (gastric emptying, gastric accommodation, and maximum tolerated volume in nutrient drink test) vs. the particular polymorphisms will be explored using multiple linear regression analyses. The physiologic responses will be considered as the dependent variables in these models and tests of the partial R^2-values for the polymorphisms adjusting for gender, BMI and race will be conducted.

Sample Size

We anticipate approximately 55% of Caucasian patients with functional dyspepsia will be CC (based on our pilot data) (100,101), and 35% will be LL (based on studies in IBS) (79). The table below lists the power for a two-sample comparison of the proportions of patients with global symptom relief (those with a specific polymorphism vs. those without). Within each treatment group (N=130), it is assumed that 55% will be CC and 35% will be LL providing sample sizes of N=70 vs. 60 and 45 vs. 85, respectively. The power listed below to detect several possible rates of global symptom relief is based on a two-sample test for proportions using a two-sided alpha level of 0.017 (i.e., adjusted for separate tests within each of the 3 treatment groups).

			Power (%)	
Relief rate in Group 1*	Relief rate in Group 2*	OR	N=70 vs. N=60	N=45 vs. N=85
15%	35%	3.0	61	57
15%	40%	3.8	81	77
15%	45%	4.6	93	90
20%	40%	2.7	55	50
20%	45%	3.3	76	71
20%	50%	4.0	90	87
25%	45%	2.5	51	46
25%	50%	3.0	72	67
25%	55%	3.7	88	84

*e.g. CC (Group 1) vs. TT/TC (Group 2), or separately LL (Group 1) vs. SS/SL (Group 2); OR = odds ratio

Hence, the study will have good power for the anticipated difference of 25-30% (e.g. 15% versus 45%). Moreover, a logistic regression model with global symptom relief as the binary dependent variable and treatment group, gender, race, BMI and polymorphism status as predictor variables should provide somewhat better power to detect corresponding associations between genotype and symptom relief by including all subjects in the same analysis assuming homogeneous associations across treatment groups.

Quality control and data management

Mayo Clinic has established the Mayo Alliance for Clinical Trials (MACT) center in Rochester, for infrastructure support for multicenter trials. To ensure the study runs effectively, and in collaboration with MACT, a number of steps have been taken:

1. Standardized outcome measures will be applied: see above
2. Data management manual: a detailed manual will be prepared listing all staff responsibilities, staff training, coordination between sites, quality control, handling of reports and blood test results, data coding and entry, data access restrictions, protocol for contingencies, and preparation of progress reports.
3. Training and study monitoring: standardized training will be given to ensure consistency across all sites. This will include a) all personnel undergoing an identical one day training session with Dr. Talley in Rochester, b) ongoing consultation, with regular at least monthly calls between physicians, study coordinators and Dr Talley, and c) standardized monitoring including adverse reactions.
4. Data collection and entry: comprehensive Case Report Forms (CRFs) will be copied and sent to Mayo Rochester where data will be reviewed and missing data will result in conversation with the study site to obtain levels or assure that data are missing. Information professionally entered. Data values outside expected ranges will be assessed and the original data sources evaluated for errors. Additionally, quality of data entry will be assessed through double data entry of selected files.

5. Data management: consistency checks will be routine across forms and visits. Data files will be backed up regularly. The Division of Biostatistics has extensive infrastructure to support the data collection, entry, and management needs of the proposed trial. This includes a data entry and management group consisting of a supervisor, four data entry clerks, and four data librarians responsible for the transfer of data from almost any electronic format to our IBM mainframe and UNIX systems for data analysis and archiving. Our archiving facility retains study data in perpetuity and assures long term access. The current location of any of over 8,000 studies undertaken since 1966 can be viewed on an on-line catalogue and retrieved within 24 hours. These efforts are supported by the Statistical Systems Section of the Department of Information Services, which comprises 32 FTE systems analyst/programmers devoted to the development and maintenance of our databases and web based applications. The Division of Information Services in the Department of Systems Support Services includes 450 additional personnel who support Mayo Clinic's information environment. The extensive computer resources at the Mayo Clinic include the Mayo Clinic Central Computer Facility, the Research Computer Facility, and multiple smaller units, all part of a network. The Mayo Clinic Central Computer Facility includes two IBM EMOS 9672-R44 and one 3090-600J mainframes, with 7GB of RAM and more than 1 terabyte of magnetic storage. Large Storagetek robotic tale silos augment nearline storage. A suite of HP UNIX database machines support Electronic Medical Record Sybase functions, in addition to fault tolerant tandem computers. These mainframe resources are complemented by a network containing SUN Enterprise class dataservers, Auxpex network fileservers, and three score SUN SPARC 20 and Ultra2 class workgroup servers within the Department of Health Sciences Research. In addition to the Central Computer Facility and the department network, Mayo also maintains a Research Computer Facility comprised of DEC VAX, SUN and Silicon Graphics machines connected to a large capacity optical jukebox fileserver. This cluster has fiberoptic router interface to the departmental network.
6. Study monitoring reports: weekly reports will be provided and monthly reports produced summarizing recruitment, compliance, errors, as well as protocol deviations, changes and clarifications and other relevant information.

Governance and oversight of the trial

Executive Committee

An Executive Committee will be responsible for oversight and management of the trial and will make and implement decisions regarding the management of the trial. The executive committee will set the agenda for the Steering Committee meetings. Drs. Talley, Locke, Zinsmeister, and Robuck, NIDDK project scientist, are the voting members on the executive committee. The executive committee will meet on a regular basis on a schedule agreed upon by the committee.

Steering Committee

A Steering Committee consisting of the principal clinical investigator for each clinical site plus all members of the Executive Committee will meet on a regular basis on a schedule agreed upon by the committee. The steering committee will provide input into the planning and conduct of the trial and will recommend changes in the protocol or data collection and analysis plans as needed. Each clinical site has one vote on the steering committee; other voting members will include the DCC PI (Dr. Zinsmeister) and the NIDDK project scientist (Dr. Robuck).

Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) has been set up in accordance with NIH policy. The DSMB is governed by and operates according to the DSMB Charter (attached).

Potential Pitfalls and Precautions Taken

1. Feasibility. The study proposes to screen approximately 1000 patients over nearly 5 years (approximately 35 patients per center per year) and randomize and follow-up 400 patients (approximately 14 patients per center per year). We already have a database of around 600 patients fully evaluated in our Mayo practice in the past 5 years in Rochester. We have published experience with all methods to be used, and have carefully considered feasibility issues when developing the study protocol to ensure complete fulfillment of all the study aims.

2. Collaborating centers may have difficulty recruiting participants. We do not envisage problems. We have six centers staffed by interested experts that will allow for the recruitment of a representative sample of patients seen in GI practice, including Black, Hispanic and Asian patients. We have extensive experience in multi-center studies including major US clinical trials. The 5 year time frame is realistic in terms of complete recruitment, intensive evaluation and follow-up.

3. Patients may not be willing to participate in the studies. We have committed that we would offer consultation and evaluation using tests, including new tests of gastric function, free of charge, and compensation for time spent participating in the study. Based on our experience, we do not expect recruitment to be an issue.

4. Blinding: Every effort will be incorporated to maintain strict blinding. The approach of instructing patients not to discuss side effects (or where known in psychological treatment trials type of therapy) is established to work effectively in our trial experience and the literature (145). Patients will be advised of all potential drug side effects in the consent form, but subject to IRB approval not each named drug's side effect profile to further reduce bias. We will also build in a standard question by the study coordinator asking the patient to guess which therapy they have been taking at week 12 to evaluate if un-blinding is an issue, but based on previous published experience in functional dyspepsia expect this not to be the case (113).

5. Managing side effects: Experienced gastroenterologists will be available for the management of problems at each site. The drugs chosen and doses selected should minimize this problem.

6. Choice of drugs and doses for testing: Very considerable thought was given to choosing the most relevant comparators. We chose amitriptyline because a) our pilot data suggest efficacy is likely and the very limited literature is consistent b)the half-life is suitable for the planned physiological studies; c) it has a relatively good side effect profile at lower dose; and d) it affects serotonin reuptake which should enhance interpretation of the pharmacogenetic studies. We have tested 25 mg and 50 mg in our pilot studies; the 50 mg dose is well tolerated. A lower dose risks missing efficacy in terms of symptom improvement, while higher doses are used for depression and would increase drop outs. We will start at a lower dose (25mg) initially for 2 weeks to maximize tolerance for the planned dose to be tested. It is conceivable a small subset will respond better to a higher antidepressant dose of amitriptyline but we would still expect to see partial response on the dose chosen, a multidose study is impractical and we specifically aim to test low dose tricyclic therapy. We chose escitalopram because a) it like citalopram is one of the most selective SSRI's, which should reduce side effects and enhance interpretation of the physiological and pharmacogenetic studies; b) it is not a racemic mixture like fluoxetine; c) citalopram has visceral analgesic actions at least in the esophagus (68); and d) the dose to be used is accepted to be standard and can be administered once daily.

7. Heterogeneity: We anticipate the response to antidepressants will be heterogeneous. We will specifically test for first and second order interactions including by gender, race, symptom subgroup, abnormal gastric emptying, abnormal satiety testing, BMI and polymorphisms. We will also explore more complex interactions if indicated by the initial analyses.

8. Race and Ethnicity: We will make very active efforts to recruit African Americans (and anticipate conservatively 20%) as well as American Indian or Alaska Native, Asian, Native Hawaiian or Pacific Islander patients. Data will be analyzed by race. Ethnicity will also be identified a Hispanic/Latino or not Hispanic/Latino. The sites have been chosen to ensure an appropriate patient mix is feasible in the setting of motility expertise.

9. Dropouts: We have assumed dropouts will occur but will provide every assistance to patients to maximize continuation. The dropout rate on desipramine in IBS in higher doses was 22% (47); we expect a lower rate but have conservatively estimated 25% in planning the trial. We plan to collect maximum data on dropouts where patients agree, including physiological testing, where applicable.
10. Gastric accommodation study: A subset will undergo this testing, and hence potential interactions will only be able to be addressed preliminarily. We are however adequately powered for the main outcomes of interest in this sub-study, which will provide novel data. We have performed 3 similar mechanistic studies, each with 20-30 IBS patients in the past 3 years. Hence, the planned studies are eminently feasible.
11. Nutrient drink test: Interpretation of this test is potentially limited because an abnormal result may occur because of impaired fundic relaxation and abnormal volume distribution, or gastric hypersensitivity, or both, and this is unknown. However, the data in the gastric accommodation sub-study will allow us to determine if the response to the drink test is associated with abnormal fundic relaxation or not; hence, we will be able to interpret the findings in the remainder of the patients under study. In the future, invasive barostat studies may then be required to evaluate the issue in further detail.
12. Collection of DNA: This research and future work on the genetic control of functional GI disorders is in accordance with Federal recommendations on the consent process. We have had a high success rate in collecting DNA samples from IBS patients. Two hundred patients and 100 healthy controls have recently consented to provide DNA and to answer a symptom questionnaire in IBS. We expect similar success in functional dyspepsia.
13. Identification of polymorphisms: We have experience in the molecular techniques planned, and in the analysis of the data. Biostatistical expertise in genetic studies is a core facility in the Department of Health Sciences Research at Mayo. Dr. Zinsmeister will utilize this resource for statistical genetics consultation. Members of the Statistical Genetics Team include Mariza de Andrade, Ph.D., Steve Iturria, Ph.D., Shane Pankratz, Ph.D., Daniel J. Schaid, Ph.D. and Terry Therneau, Ph.D. Specialized statistical genetics software packages installed from the Rockefeller web site and genetic analysis software written in SAS, S-PLUS, and stand-alone packages are available.
14. Selection of polymorphisms: We are well aware that many other polymorphisms than those proposed have been described in the literature. Our choice of polymorphisms was based on a comprehensive review, with increased weight given to the published literature when functional studies were linked to the description of the polymorphism. We will have ready access to the stored samples and can reanalyze them if required. Specifically, if no C825T polymorphism association is found, which appears to be unlikely given our provocative preliminary data, we will initially screen for the C1429T polymorphism that has been shown to be in tight linkage disequilibrium to C825T.

III. Human Subjects

Subjects

Adult patients with functional dyspepsia will be recruited, without restrictions for gender, race or ethnic group. Clinic patients and media advertising will be sources of patients; the referral pathway will be recorded and considered in the analyses. We anticipate that among patients there will be a majority of whites, at least 20% African Americans and a small percentage of Hispanics and Asians, consistent with the ethnic constitution of communities in the proposed sites. We will also increase minority recruitment actively by involving local physicians who look after their patients in the community. In the setting of research to test the efficacy of screening colonoscopy for example, recruitment of ethnic minorities was increased once local physicians were involved and made the recommendation (146). We will randomize 400 patients aged 18- 75 (mean age 40 in past studies). The ratio of females to males in functional dyspepsia is 1 to 1.5 females to 1 male; our

randomization strategy will ensure an equal sex distribution. There are no a priori data suggesting women respond differently from men with functional dyspepsia to pharmacologic intervention although this has been seen in IBS (45). Children, 18-21 years of age, will be eligible for participation in the study. It is inappropriate to consider younger children because it is unknown if antidepressant therapy is efficacious in adults. Exclusion criteria will be applied to maximize participant safety.

All subjects will be given a verbal explanation of the study, provided time to read and study the written consent form and its information, given opportunities to ask questions and a copy of the consent form. Participants will be informed of their right to withdraw from the study at any time without prejudice to their clinical management now or in the future. Consent will be sought by one of the medical physician investigators or study coordinator, and consent will be documented by the participant's signature on the consent form. Specific information will be provided in the consent form regarding storage and future use of the DNA sample (147).

Participants will be recruited from the clinical practice of the investigator. Patients seen in the past at Mayo Clinic will also be contacted via posted ads and recruitment letter. The following ad will be used for study advertisement at the Rochester site.

Researchers at Mayo Clinic are studying the effects of two FDA-approved medications to treat stomach symptoms that occur after eating. In this study volunteers who experience upper abdominal pain or discomfort, bloating, nausea and/or fullness after eating a meal are being asked to participate. All study-related examinations, tests and medications are provided at no charge to participants, the National Institutes of Health are sponsoring the study.
Details: For more information, please call Vickie Silvernail at 507-284-2812.

At all participating sites, the local investigator will apply to the local Institutional Review Board/Ethics committee for approval of the trial and genotyping studies.

Human Safety Issues

Prior to initiating the study, all subjects will provide written informed consent using forms approved by the Institutional Review Boards. The consent form becomes a permanent part of the medical record at Mayo Clinic. The consent form includes information relative to genetic studies and storage of genetic material for future research, and conforms to the high standards required for such studies. Since this genetic information is not yet pertinent to clinical practice, the information will not be included in the medical record, but will be maintained in a coded fashion which excludes patient identifiers.

Amitriptyline and escitalopram are in broad use in the community. Safeguards have been put in place including regular visits, and assessment of blood levels.

A data and safety-monitoring plan has been established for this trial. Reporting mechanisms will be put in place for adverse events to the IRB, the NIH and the FDA.

Responsible Conduct of Human Research

Mayo Clinic Rochester has established a formal program entitled the Mayo Investigator Training Program or MITP. The MITP is a web based educational course designed to provide all personnel involved in human subject research with training about human subject protection. All Mayo personnel engaged in human subject research are required to complete the course. The primary objectives of the course are to provide the historical framework for current human subject protection regulations and to explore the evolving issues related to human subject research. The course is divided into four sections:

- Course introduction and general overview
- History section which explores examples of unethical behavior in human subject research
- Review of major human subject protection issues

- Discussion of the various roles and responsibilities of individuals involved in human subject research

At the conclusion of the instruction, individuals are required to complete a thirty-question assessment.

All Mayo investigators have completed the Mayo IRB's mandated certification in the responsible conduct of research, and investigators elsewhere will have completed similar certification.

Source of Research Material

Research material will be the medical records (of those who authorize review of the records for research purposes), questionnaires prospectively acquired, quantitation of gastric emptying with an external gamma camera, gastric volume measurements with an external SPECT camera and venous blood samples from which DNA will be extracted and stored.

Risks and Precautions

Radiation exposure in this study includes radiation from the ^{99m}Tc Sulfur colloid used to measure gastric emptying, and the $^{99m}TcO_4^-$ for the gastric accommodation study using SPECT; the latter will be performed on two occasions, approximately 3 months apart. These exposures conform to previously approved levels of radiation exposure approved by the Radiation Control Committee at Mayo Clinic. The radiation dosimetry and organ exposures are listed below:

Dosimetry and Organ Exposure in mrad

GASTRIC EMPTYING

Radiopharmaceutical	Activity mCi	Body	Gonads	Breast	Red marrow	Lung	Thyroid	Bone	ULI	Colon	Stom-ach	Blad-der	Liv-er	Esoph-agus	Oth-er
^{99m}TcSulfur Colloid	0.5mCi	20	90	20				420	300	130	20	10	220		
GASTRIC ACCOMMODATION															
$^{99m}TcO_4^-$	10.0	130	370	70	135	95	815	200	2110	1555	965	660	1405	90	315

(mrad= radiation absorbed dose to organs)

H_e or the radiation effective dose to the body summarizes the risk to the whole body as the individual doses to each of the organs; effective dose is used to compare risks among various types of x ray and radionuclide studies:

^{99m}TcSulfur colloid 1.0 mCi H_e 90 mrem
$^{99m}TcO_4^-$ 10.0 mCi H_e 619 mrem
(mrem= radiation equivalent dose)

In view of the radiation exposure, all females of childbearing age will be required to have a negative blood pregnancy test prior to performance of the nuclear medicine studies.

In summary, the risks to the participants are reasonable since all procedures are noninvasive, precautions are taken to minimize discomfort or adverse effects, strategies are set a priori to treat adverse effects, and radiation exposure is being kept to the minimum that allows successful completion and data acquisition. There is a high likelihood (e.g., investigators' record and expertise, validated methods, statistical power) of obtaining meaningful, useful information in each specific aim. Results of the studies will be maintained in summaries included in their medical record and in computer files protected by a personal password. In the latter files, participants will be identified only by a registration number rather than by name to ensure confidentiality. Since genetic information collected is not yet pertinent to clinical practice, the information will not be included in the medical record, but will be maintained in a coded fashion which excludes patient identifiers (also excluding their identification number). Many of the principles described by Beskow et al with respect to the exploratory analysis of gene-disease associations apply (147); thus, the consent process for genetic analysis will not require exhaustive genetic counseling.

References:

1. Talley NJ, Zinsmeister AR, Schleck CD, Melton LJr. Dyspepsia and dyspepsia subgroups: a population-based study. Gastroenterology 1992;102:1259-68.
2. Locke GR. The epidemiology of functional gastrointestinal disorders in North America. Gastroenterol Clin North Am 1996;25:1-19.
3. Koloski NA, Talley NJ, Boyce PM. Epidemiology and health care seeking in the functional GI disorders: a population-based study. American Journal of Gastroenterology. 2002;97:2290-9.
4. Koloski N, Talley N, Boyce P. Predictors of health care seeking for irritable bowel syndrome and nonulcer dyspepsia: a critical review of the literature on symptom and psychosocial factors. Am J Gastroenterol 2001;96:1340-49.
5. Sandler R, Everhart J, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The burden of selected digestive diseases in the United States. Gastroenterology 2002;122:1500-11.
6. Agreus L, Borgquist L. The cost of gastro-oesophageal reflux disease, dyspepsia and peptic ulcer disease in Sweden. Pharmacoeconomics 2002;20:347-55.
7. Talley NJ, Silverstein MD, Agréus L, Nyrén O, Sonnenberg A, Holtmann G. AGA Technical Review: evaluation of dyspepsia. Gastroenterology 1998;114:582-95.
8. Talley NJ, Stanghellini V, Heading R, Koch KL, Malagelada JR, Tytgat GNJ. Functional gastroduodenal disorders: a working team report for the Rome II consensus on funtional gastrointestinal disorders. Gut 1999;45 (Suppl 2):II37-42.
9. Delaney BC, Moayyedi P, Forman D. Initial management strategies for dyspepsia. Cochrane Database of Systematic Reviews 2003;2:CD001961.
10. Veldhuyzen van Zanten SJ, Jones MJ, Verlinden M, Talley NJ. Efficacy of cisapride and domperidone in functional (nonulcer) dyspepsia: a meta-analysis. Am J Gastroenterol 2001;96:689-96.
11. Veldhuyzen van Zanten SJ, Cleary C, Talley NJ, Peterson TC, Nyren O, Bradley LA, Verlinden M, Tytgat GN. Drug treatment of functional dyspepsia: a systematic analysis of trial methodology with recommendations for design of future trials. Am J Gastroenterol 1996;91:660-73.
12. Tack J, Delia T, Ligizio G, LSue S, Lefkowitz M, Vandeplassche L. A phase II placebo controlled randomized trial with tegaserod in functional dyspepsia patients with normal gastric emptying. Gastroenterology 2002;122 (Suppl 1):A20.
13. Bensoussan A, Talley NJ, Hing M, Menzies R, Guo A, Ngu M. Treatment of irritable bowel syndrome with Chinese herbal medicine: a randomized contolled trial. JAMA 1998;280:1585-1589.
14. Calvert EL, Houghton LA, Cooper P, Morris J, Whorwell P. Long-term improvement in functional dyspepsia using hypnotherapy. Gastroenterology 2002;123:1778-1785.
15. Soo S, Moayyedi P, Deeks J, Delaney B, Lewis M, Forman D. Psychological interventions for non-ulcer dyspepsia. Cochrane Database Syst Rev 2001;(4):CD002301.
16. Mishra DN, Shukla GD, Agarwal AK, Saxena HN, Jain PK. Non-organic dyspepsia: a controlled clinico-psychiatric study. J Assoc Physicians India 1984;32:399-402.
17. Magni G, di Mario F, Bernasconi G, Mastropaolo G. DSM-III diagnoses associated with dyspepsia of unknown cause. Am J Psychiatry 1987;144:1222-3.
18. Haug TT, Wilhelmsen I, Ursin H, Berstad A. What are the real problems for patients with functional dyspepsia? Scand J Gastroenterol 1995;30:97-100.
19. Haug TT, Svebak S, Wilhelmsen I, Berstad A, Ursin H. Psychological factors and somatic symptoms in functional dyspepsia. A comparison with duodenal ulcer and healthy controls. J Psychosom Res 1994;38:281-91.

20. Quartero AO, Post MW, Numans ME, de Melker RA, de Wit NJ. What makes the dyspeptic patient feel ill? A cross sectional survey of functional health status, *Helicobacter pylori* infection, and psychological distress in dyspeptic patients in general practice. Gut 1999;45:15-9.
21. Talley NJ, Boyce P, Jones M. Dyspepsia and health care seeking in a community: How important are psychological factors? Dig Dis Sci 1998;43:1016-1022.
22. Colgan S, Creed F, Klass H. Symptom complaints, psychiatric disorder and abnormal illness behaviour in patients with upper abdominal pain. Psychol Med 1988;18:887-92.
23. Porcelli P, Taylor GJ, Bagby RM, De Carne M. Alexithymia and functional gastrointestinal disorders. A comparison with inflammatory bowel disease. Psychother Psychosom 1999;68:263-9.
24. Talley NJ, Phillips SF, Bruce B, Twomey CK, Zinsmeister AR, Melton LJ. Relation among personality and symptoms in non-ulcer dyspepsia and the irritable bowel syndrome. Gastroenterology 1990;99:327-333.
25. Lynch ME. Antidepressants as analgesics: a review of randomized controlled trials. J Psychiatry Neurosci 2001;26:30-6.
26. Mertz H, Morgan V, Tanner G, Pickens D, Price R, Shyr Y, Kessler R. Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. Gastroenterology. 2000;118:842-8.
27. Clouse RE. Antidepressants for functional gastrointestinal syndromes. Dig Dis Sci 1994;39:2352-63.
28. Clouse RE, Lustman PJ, Geisman RA, Alpers DH. Antidepressant therapy in 138 patients with irritable bowel syndrome: a five-year clinical experience. Aliment Pharmacol Ther 1994;8:409-16.
29. O'Malley PG, Jackson JL, Santoro J, Tomkins G, Balden E, Kroenke K. Antidepressant therapy for unexplained symptoms and symptom syndromes. J Fam Pract 1999;48:980-90.
30. Prakash C, Lustman PJ, Freedland KE, Clouse RE. Tricyclic antidepressants for functional nausea and vomiting: clinical outcome in 37 patients. Dig Dis Sci 1998;43:1951-6.
31. Jackson JL, O'Malley PG, Tomkins G, Balden E, Santoro J, Kroenke K. Treatment of functional gastrointestinal disorders with antidepressant medications: a meta-analysis. Am J Med 2000;108:65-72.
32. Gorelick AB, Koshy SS, Hooper FG, Bennett TC, Chey WD, Hasler WL. Differential effects of amitriptyline on perception of somatic and visceral stimulation in healthy humans. Am J Physiol 1998;G460-6.
33. Cox ID, Hann CM, Kaski JC. Low dose imipramine improves chest pain but not quality of life in patients with angina and normal coronary angiograms. Eur Heart J 1998;19:250-4.
34. Cannon RO, 3rd., Quyyumi AA, Mincemoyer R, Stine AM, Gracely RH, Smith WB, Geraci MF, Black BC, Uhde TW, Waclawiw MA, al. e. Imipramine in patients with chest pain despite normal coronary angiograms. N Engl J Med 1994;330:1411-7.
35. McQuay HJ, Moore RA. Antidepressants and chronic pain. BMJ 1997;314:763-4.
36. Collins SL, Moore RA, McQuay HJ, Wiffen P. Antidepressants and anticonvulsants for diabetic neuropathy and postherpetic neuralgia: a quantitative systematic review. J Pain Symptom Manage 2000;20:449-58.
37. Varia I, Logue E, O'connor C, Newby K, Wagner HR, Davenport C, Rathey K, Krishnan KR. Randomized trial of sertraline in patients with unexplained chest pain of noncardiac origin. Am Heart J. 2000 2000;140:369-72.
38. Anderberg UM, Marteinsdottir I, von Knorring L. Citalopram in patients with fibromyalgia--a randomized, double-blind, placebo-controlled study. Eur J Pain 2000;4:27-35.
39. Chang L. The association of functional gastrointestinal disorders and fibromyalgia. Eur J Surg Suppl. 1998;(583):32-6. 1998;(583):32-6.
40. Tack JF, Vox R, Broekaert D, Fischler B, Janssens J. Influence of citalopram, a selective serotonin reuptake inhibitor, on colonic tone and sensitivity in man. Gastroenterology 2000;118:A174.

41. Broekaert D, Vos R, Gevers A-M, Janssens J, Vandenberghe J, Fischler B, Tack J. A double-blind randomized placebo-controlled crossover trial of citalopram, a selective 5-hydroxytryptamine reuptake inhibitor in irritable bowel syndrome. Gastroenterology 2001;120:A641.
42. Chial HJ, Camilleri M, Ferber I, Delgado-Aros S, Burton D, McKinzie S, Zinsmeister AR. Effects of venlafaxine, buspirone, and placebo on colonic sensorimotor functions in healthy humans. Clinical Gastroenterology and Hepatology 2003;1:211-218.
43. O'Malley PG, Balden E, Tomkins G, Santoro J, Kroenke K, Jackson JL. Treatment of fibromyalgia with antidepressants: a meta-analysis. J Gen Intern Med 2000;15:659-66.
44. Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, Gelenberg AJ, Davis SM, Harrison WM, Keller MB. Gender differences in treatment response to sertraline versus imipramine in chronic depression. Am J Psychiatry 2000;157:1445-52.
45. Talley NJ. Serotoninergic neuroenteric modulators. Lancet 2001;358:2061-2068.
46. Talley N, Spiller RC. Irritable bowel syndrome: A little understood organic bowel disease? Lancet 2002;360:555-564.
47. Drossman D, Toner BB, Whitehead WE, Diamant NE, Dalton CB, Duncan S, Emmott S, Proffitt V, Akman D, Frusciante K, Le T, Meyer K, Bradshaw B, Mikula K, Morris CB, Blackman CJ, Hu Y, Jia H, Li JZ, Koch GG, Bangdiwala SI. Cognitive-behavioral therapy versus education and desipramine versus placebo for moderate to severe functional bowel disorders. Gastroenterology 2003;125:19-31.
48. Mertz H, Fass R, Kodner A, Yan-Go F, Fullerton S, Mayer EA. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. Am J Gastroenterol 1998;93:160-65.
49. Tanum L, Malt UF. A new pharmacologic treatment of functional gastrointestinal disorder. A double-blind placebo-controlled study with Mianserin. Scand J Gastroenterol 1996;31:318-25.
50. Tanum L, Malt UF. Personality traits predict treatment outcome with an antidepressant in patients with functional gastrointestinal disorder. Scand J Gastroenterol 2000;35:935-41.
51. Kuiken SD, Tytgat GN, Boeckxstaens GE. The selective serotonin reuptake inhibitor fluoxetine does not change rectal sensitivity and symptoms in patients with irritable bowel syndrome: a double blind, randomized, placebo-controlled study. Clin Gastoenterol Hepatol 2003;1:219-228.
52. Bytzer P, Hallas J. Drug-induced symptoms of functional dyspepsia and nausea. A symmetry analysis of one million prescriptions. Aliment Pharmacol Ther 2000;14:1479-84.
53. Talley NJ, Meineche-Schmidt V, Pare P, Duckworth M, Raisanen P, Pap A, Kordecki H, Schmid V. Efficacy of omeprazole in functional dyspepsia: double-blind, randomized, placebo-controlled trials (the Bond and Opera studies). Aliment Pharmacol Ther 1998;12:1055-1065.
54. Wong WM, Wong BC, Hung WK, Yee YK, Yip AW, Szeta ML, Fund FM, Tong TS, Lai KC, Hu WH, Yuen MF, Lam SK. Double blind, randomised, placebo controlled study of four weeks of lansoprazole for the treatment of functional dyspepsia in Chinese patients. Gut 2002;51:502-506.
55. Stanghellini V, Tosetti C, Paternic'o A, Barbara G, Morselli-Labate AM, Monetti N, Marengo M, Corinaldesi R. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996;110:1036-42.
56. Quartero AO, de Wit NJ, Lodder AC, Numans ME, Smout AJ, Hoes AW. Disturbed solid-phase gastric emptying in functional dyspepsia: a meta-analysis. Dig Dis Sci 1998;43:2028-33.
57. Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998;115:1346-52.
58. Holtmann G, Gschossmann J, Neufang-Huber J, Gerken G, Talley NJ. Differences in gastric mechanosensory function after repeated ramp distensions in non-consulters with dyspepsia and healthy controls. Gut. 2000;47:332-6.
59. Tack J, Caenepeel P, Fischler B, Piessevaux H, Janssens J. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. Gastroenterology 2001;121:526-35.

60. Stanghellini V, Tosetti C, Paternic'o A, De Giorgio R, Barbara G, Salvioli B, Corinaldesi R. Predominant symptoms identify different subgroups in functional dyspepsia. Am J Gastroenterol 1999;94:2080-85.
61. Talley NJ, Verlinden M, Jones M. Can symptoms discriminate among those with delayed or normal gastric emptying in dysmotility-like dyspepsia? Am J Gastroenterol 2001;96:1422-28.
62. Boeckxstaens G, Hirsch D, Kuiken S, Heisterkamp S, Tytgat G. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002;97:40-8.
63. Tack J, Coulie B, Wilmer A, Andrioli A, Janssens J. Influence of sumatriptan on gastric fundus tone and on the perception of gastric distension in man. Gut 2000;46:468-73.
64. Gorard DA, Libby GW, Farthing MJG. Influence of antidepressants on whole gut orocaecal transit times in health and irritable bowel syndrome. Aliment Pharmacol Ther 1994;8:159-166.
65. Ladabaum U, Glidden D. Effect of the selective serotonin reuptake inhibitor sertraline on gastric sensitivity and compliance in healthy humans. Neurogastroenterol Motil 2002;14:395-402.
66. Tack J, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. Aliment Pharmacol Ther 2003;17:603-8.
67. Chial HJ, Camilleri M, Burton D, Thomforde G, Olden KW, Stephens D. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am J Physiol Gastrointest Liver Physiol 2003;284:G130-7.
68. Broekaert D, Vos R, Sifrim D. The influence of citalopram, a selective serotonin reuptake inhibitor, on esophageal sensitivity and motility in man. Gastroenterology 2002;120:3192 (Abstract).
69. Liau SS, Camilleri M, Kim DY, Stephens D, Burton DD, O'Connor MK. Pharmacological modulation of human gastric volumes demonstrated noninvasively using SPECT imaging. Neurogastroenterol Motil 2001;13:533-42.
70. Kim DY, Delgado-Aros S, Camilleri M, Samsom M, Murray JA, O'Connor MK, Brinkmann BH, Stephens DA, Lighvani SS, Burton DD. Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. Am J Gastroenterol 2001;96:3099-105.
71. Chial HJ, Camilleri C, Delgado-Aros S, Burton D, Thomforde G, Ferber I, Camilleri M. A nutrient drink test to assess maximum tolerated volume and postprandial symptoms: effects of gender, body mass index and age in health. Neurogastroenterology and Motility 2002;14:249-53.
72. Locke GR, III., Zinsmeister AR, Talley NJ, Fett SL, Melton LJ, III. Familial association in adults with functional gastrointestinal disorders. Mayo Clin Proc 2000;75:907-912.
73. Lembo T, Zaman MS, Chavez NF, Crueger R, Jones MP, Talley NJ. Concordance of IBS among monzygoitc and dizygotic twins. Gastroenterology 2001;120 (Suppl 1):A66.
74. Morris-Yates A, Talley NJ, Boyce PM, Nandurkar S, Andrews G. Evidence of a genetic contribution to functional bowel disorder. Am J Gastroenterol 1998;93:1311-1317.
75. Levy RL, Jones KR, Whitehead WE, Feld SI, Talley NJ, Corey LA. Irritable bowel syndrome in twins: heredity and social learning both contribute to etiology. Gastroenterology 2001;121:799-804.
76. Lerer B, Macciardi F. Pharmacogenetics of antidepressant and mood-stabilizing drugs: a review of candidate-gene studies and future research directions. Int J Neuropsychopharmacol 2002;5:255-75.
77. Blakely RD. Physiological genomics of antidepressant targets: keeping the periphery in mind. J Neurosci 2001;21:8319-23.
78. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527-31.

79. Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, McKinzie S, Urrutia R. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002;123:425-32.
80. Lotrich FE, Pollock BG, Ferrell RE. Polymorphism of the serotonin transporter: implications for the use of selective serotonin reuptake inhibitors. Am J Pharmacogenomics 2001;1:153-64.
81. Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G, Wittig B. Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. Nature 1991;353:43-8.
82. Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B. Different beta-subunits determine G-protein interaction with transmembrane receptors. Nature 1992;358:424-6.
83. Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B. Selectivity in signal transduction determined by gamma subunits of heterotrimeric G proteins. Science 1993;259:832-4.
84. Baumgart D, Naber C, Haude M, Oldenburg O, Erbel R, Heusch G, Siffert W. G protein beta3 subunit 825T allele and enhanced coronary vasoconstriction on alpha(2)-adrenoceptor activation. Circ Res 1999;85:965-9.
85. Zill P, Baghai TC, Zwanzger P, Schule C, Minov C, Riedel M, Neumeier K, Rupprecht R, Bondy B. Evidence for an association between a G-protein beta3-gene variant with depression and response to antidepressant treatment. Neuroreport 2000;11:1893-7.
86. Lin CN, Tsai SJ, Hong CJ. Association analysis of a functional G protein beta3 subunit gene polymorphism (C825T) in mood disorders. Neuropsychobiology 2001;44:118-21.
87. Virchow S, Ansorge N, Rubben H, Siffert G, Siffert W. Enhanced fMLP-stimulated chemotaxis in human neutrophils from individuals carrying the G protein beta3 subunit 825 T-allele. FEBS Lett 1998;436:155-158.
88. Lindemann M, Virchow S, Ramann F, Barsegian V, Kreuzfelder E, Siffert W, Muller N, Grosse-Wilde H. The G protein beta3 subunit 825T allele is a genetic marker for enhanced T cell response. FEBS Lett 2001;495:82-86. FEBS Lett 2001;495:82-86.
89. Bharucha AE, Camilleri M, Zinsmeister AR, Hanson RB. Adrenergic modulation of human colonic motor and sensory function. Am J Physiol 1997;273:G997-1006.
90. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. Hypertension 2001;37:739-73.
91. Siffert W, Forster P, Jockel KH, Mvere DA, Brinkmann B, Naber C, Crookes R, Du P, Heyns A, Epplen JT, Fridey J, Freedman BI, Muller N, Stolke D, Sharma AM, Al Moutaery K, Grosse-Wilde H, Buerbaum B, Ehrlich T, Ahmad HR, Horsthemke B, Du Toit ED, Tiilikainen A, Ge J, Wang Y, Rosskopf D. Worldwide ethnic distribution of the G protein beta3 subunit 825T allele and its association with obesity in Caucasian, Chinese, and Black African individuals. J Am Soc Nephrol 1999;10:1921-30.
92. Talley NJ, Phillips SF, Melton J, 3rd, Wiltgen C, Zinsmeister AR. A patient questionnaire to identify bowel disease. Ann Intern Med 1989;111:671-4.
93. Talley NJ, Moore MG, Sprogis A, Katelaris P. Randomised controlled trial of pantoprazole versus ranitidine for the treatment of uninvestigated heartburn in primary care. Medical Journal of Australia 2002;177:423-7.
94. Talley NJ, Verlinden M, Jones M. Validity of a new quality of life scale for functional dyspepsia: a United States multicenter trial of the Nepean Dyspepsia Index. Am J Gastroenterol 1999;94:2390-7.
95. Talley NJ, Haque M, Wyeth JW, Stace NH, Tytgat GN, Stanghellini V, Holtmann G, Verlinden M, Jones M. Development of a new dyspepsia impact scale: the Nepean Dyspepsia Index. Alimentary Pharmacology & Therapeutics 1999;13:225-35.
96. Talley NJ, Fullerton S, Junghard O, Wiklund I. Quality of life in patients with endoscopy-negative heartburn: reliability and sensitivity of disease-specific instruments. Am J Gastroenterol 2001;96:1998-2004.

97. Kuiken SD, Samsom M, Camilleri M, Mullan BP, Burton DD, Kost LJ, Hardyman TJ, Brinkmann BH, O'Connor MK. Development of a test to measure gastric accommodation in humans. Am J Physiol 1999;277 (6 Pt 1):G1217-21.
98. Bouras EP, Delgado-Aros S, Camilleri M, Castillo EJ, Burton DD, Thomforde GM, Chial HJ. SPECT imaging of the stomach: comparison with barostat, and effects of sex, age, body mass index, and fundoplication. Single photon emission computed tomography. Gut 2002;51:781-6.
99. Bruley des Varannes S, Parys V, Ropert A, Chayvialle JA, Roze C, Galmiche JP. Erythromycin enhances fasting and postprandial proximal gastric tone in humans. Gastroenterology 1995;109:32-9.
100. Holtmann G, Siffert W, Haag S, Mueller N, Langkafel M, Senf W, Zotz R, Talley NJ. G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. Gastroenterology 2004;126:971-79.
101. Holtmann G, Grote E, Braun-Lang U, Haag S, Pietsch A, Talley NJ, Siffert W. G-protein β 3 subunit (GNβ3) 825 CC genotype and the manifestation of functional gastrointestinal disorders. Gastroenterology 2004;126 (Suppl 2):A-162.
102. Holtmann G, Siffert W, Grote E, Haag S, Braun-Land U, Langkafel M, Talley NJ. G-protein mediated receptor cell-coupling as a predictor for the long term response to treatment in patients with functional dyspepsia. Gastroenterology 2003;18 Suppl:B96.
103. Kim HJ, Camilleri M, Carlson PJ, Cremonini F, Ferber I, Stephens D, McKinzie S, Zinsmeister AR, Urrutia R. Association of distinct alpha(2) adrenoceptor and serotonin transporter polymorphisms with constipation and somatic symptoms in functional gastrointestinal disorders. Gut 2004;53:829-37.
104. Veldhuyzen van Zanten SJ, Talley NJ, Bytzer P, Klein KB, Whorwell PJ, Zinsmeister AR. Design of treatment trials for functional gastrointestinal disorders. Gut 1999;45 (Suppl 2):II69-77.
105. Talley NJ, Vakil N, Ballard ED, Fennerty MB. Absence of benefit of eradicating *Helicobacter pylori* in patients with nonulcer dyspepsia. N Eng J Med 1999;341:1106-1111.
106. Blum AL, Talley NJ, O'Morain C, van Zanten SV, Labenz J, Stolte M, Louw JA, Stubberod A, Theodors A, Sundin M, Bolling-Sternevald E, Junghard O. Lack of effect of treating Helicobacter pylori infection in patients with nonulcer dyspepsia. N Engl J Med 1998;339:1875-1881.
107. Talley NJ, Janssens J, Lauritsen K, Racz I, Bolling-Sternevald E. Eradication of *Helicobacter pylori* in functional dyspepsia: randomised double blind placebo controlled trial with 12 months' follow up. The Optimal Regimen Cures *Helicobacter* Induced Dyspepsia (ORCHID) Study Group. BMJ 1999;318:833-837.
108. Talley NJ, Venables TL, Green JR, Armstrong D, O'Kane KP, Giaffer M, Bardhan KD, Carlsson RG, Chen S, Hasselgren GS. Esomeprazole 40 mg and 20 mg is efficacious in the long-term management of patients with endoscopy-negative gastro-oesophageal reflux disease: a placebo-controlled trial of on-demand therapy for 6 months. European Journal of Gastroenterology & Hepatology 2002;14:857-63.
109. Talley NJ, Chang FY, Wyatt JM, Adams S, Lau A, Borody T, Chen TS, Daskolopolos G, Cheung K, Talley NA. Nizatidine in combination with amoxycillin and clarithromycin in the treatment of *Helicobacter pylori* infection. Aliment Pharmacol Ther 1998;12:527-32.
110. Talley NJ, Verlinden M, Snape WJ, Beker JA, Ducrotte P, Dettmer A, Brinkhoff H, Eaker EY, Ohning G, Miner PB, Jr., Mathias JR, Fumagalli I, Staessen D, Mack RJ. Failure of a motilin receptor agonist (ABT-229) to relieve the symptoms of functional dyspepsia in patients with and without delayed gastric emptying: a randomized double-blind placebo-controlled trial. Aliment Pharmacol Ther 2000;14:1653-61.
111. Talley NJ, Lauritsen K, Tunturi-Hihnala H, Lind T, Moum B, Bang C, Schulz T, Omland TM, Delle M, Junghard O. Esomeprazole 20 mg maintains symptom control in endoscopy-negative gastro-oesophageal reflux disease: a controlled trial of 'on-demand' therapy for 6 months. Aliment Pharmacol Ther 2001;15:347-54.

112. Meineche-Schmidt V, Talley NJ, Pap A, Kordecki H, Schmid V, Ohlsson L, Wahlqvist P, Wiklund I, Bolling-Sternevald E. Impact of functional dyspepsia on quality of life and health care consumption after cessation of antisecretory treatment. A multicentre 3-month follow-up study. Scand J Gastroenterol 1999;34:566-74.
113. Talley NJ, McNeil D, Hayden A, Piper DW. Randomized, double-blind, placebo-controlled crossover trial of cimetidine and pirenzepine in nonulcer dyspepsia. Gastroenterology 1986;91:149-56.
114. Steadman CJ, Talley NJ, Phillips SF, Zinsmeister AR. Selective 5-hydroxytryptamine type 3 receptor antagonism with ondansetron as treatment for diarrhea-predominant irritable bowel syndrome: a pilot study. Mayo Clin Proc 1992;67:732-8.
115. Katelaris PH, Forbes GM, Talley NJ, Crotty B. A randomized comparison of quadruple and triple therapies for *Helicobacter pylori* eradication: The QUADRATE Study. Gastroenterology 2002;123:1763-9.
116. Talley NJ, Verlinden M, Geenen DJ, Hogan RB, Riff D, McCallum RW, Mack RJ. Effects of a motilin receptor agonist (ABT-229) on upper gastrointestinal symptoms in type 1 diabetes mellitus: a randomised, double blind, placebo controlled trial. Gut 2001;49:395-401.
117. Talley NJ, Riff DS, Schwartz H, Marcuard SP. Double-blind placebo-controlled multicentre studies of rebamipide, a gastroprotective drug, in the treatment of functional dyspepsia with or without *Helicobacter pylori* infection. Aliment Pharmacol Ther 2001;15:1603-11.
118. Holtmann G, Gschossmann J, Mayr P, Talley NJ. A randomised placebo-controlled trial of simethicone and cisapride for the treatment of patients with functional dyspepsia. Aliment Pharmacol Ther 2002;16:1641-1648.
119. Moher D, Schulz KF, Altman DG, CONSORT GROUP. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. Ann Intern Med 2001;134:657-62.
120. Tack J, Talley NJ, Camilleri M, Holtmann G, Malagelada J, Stanghellini V. Functional gastroduodenal disorders. Gastroenterology 2006;130:1466-79.
121. Boyer EW, Shannon M. The serotonin syndrome. N Engl J Med 2005;352:1112-20.
122. Therneau T. How many stratification factors are "too many" to use in a randomization plan? Control Clin Trials 1993;14:98-108.
123. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975;31:103-15.
124. Kalish LA, CB B. The impact of treatment allocation procedures on nominal significance levels and bias. Control Clin Trials 1987;8:121-35.
125. Junghard O, Lauritsen K, Talley NJ, Wiklund IK. Validation of seven graded diary cards for severity of dyspeptic symptoms in patients with non ulcer dyspepsia. Eur J Surg Suppl 1998;(583):106-11.
126. Wiklund IK, Junghard O, Grace E, Talley NJ, Kamm MA, Veldhuyzen van Zanten S, Pare P, Chiba N, Leddin DS, Bigard MA, Colin R, Schoenfeld P. Quality of life in reflux and dyspepsia patients. Psychometric documentation of a new disease-specific questionnaire (QOLRAD). Eur J Surg Suppl 1998;(583):41-9.
127. Fawcett J, Epstein P, Fiester SJ, Elkin I, Autry JH. Clinical management--imipramine/placebo administration manual. NIMH Treatment of Depression Collaborative Research Program. Psychopharmacol Bull 1987;23:309-24.
128. Talley NJ, Van Zanten SV, Saez LR, Dukes GE, Perschy T, Heath M, Kleoudis C, Mangel AW. A dose-ranging, placebo-controlled, randomized trial of alosetron in patients with functional dyspepsia. Aliment Pharmacol Ther 2001;15:525-37.

129. Camilleri M, Chey WY, Mayer EA, Northcutt AR, Heath A, Dukes GE, McSorley D, Mangel AM. A randomized controlled clinical trial of the serotonin type 3 receptor antagonist alosetron in women with diarrhea-predominant irritable bowel syndrome. Arch Intern Med 2001;161:1733-1740.
130. Lembo T, Wright RA, Bagby B, Decker C, Gordon S, Jhingran P, Carter E, Team LI. Alosetron controls bowel urgency and provides global symptom improvement in women with diarrhea-predominant irritable bowel syndrome. Am J Gastroenterol 2001;96:2662-2670.
131. Locke GR, III, Weaver AL, Melton LJ, III, Talley NJ. Psychosocial factors are linked to functional gastrointestinal disorders: A population based nested case-control study. Am J Gastroenterol 2004;99:350-57.
132. Kalantar JS, Locke GR, III, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. Gut 2003;52:1703-07.
133. Boyce PM, Talley NJ, Balaam B, Koloski NA, Truman G. A randomized controlled trial of cognitive behavior therapy, relaxation training, and routine clinical care for the irritable bowel syndrome. Am J Gastroenterol 2003;98:2209-2218.
134. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.
135. McNair DM, Loor M, Droppleman LF. Profile of Mood States Manual. Multi Health Systems, 2003.
136. Speilberger CD, Gorusch RI, Luschene RE. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, 1970.
137. Welstein L, Dement DC, Redington D, Guillemnault C. Insomnia in the San Francisco Bay Area: A Telephone Survey. Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution. New York: Raven Press, 1983:73-85.
138. Drossman DA, Li Z, Toner BB, Diamant NE, Creed FH, Thompson D, Read NW, Babbs C, Barreiro M, Bank L, et al. Functional bowel disorders. A multicenter comparison of health status and development of illness severity index. Dig Dis Sci 1995;40:986-95.
139. Ware JE, Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.
140. Bensoussan A, Chang SW, Menzies RG, Talley NJ. Application of the general health status questionnaire SF36 to patients with gastrointestinal dysfunction: initial validation and validation as a measure of change. Aust N Z J Public Health 2001;25:71-7.
141. Tougas G, Eaker EY, Abell T, Abrahamsson H, Boivin M, Chen J, Hocking MP, Quigley EM, Koch KL, Tokayer AZ, Stanghellini V, Chen Y, Huizinga JD, Ryden J, Bourgeois IM, McCallum R. Assessment of gastric emptying using a low fat meal: establishment of international control values. Am J Gastroenterol 2000;95:1456-62.
142. Degnen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. Gut 1996;39:299-305.
143. Rosskopf D, Busch S, Manthey I, Siffert W. G protein beta 3 gene: structure, promoter, and additional polymorphisms. Hypertension 2000;36:33-41.
144. Hauck WW, Anderson S, Marcus SM. Should we adjust for covariates in nonlinear regression analyses of randomized trials? Control Clin Trials 1998;19:249-56.
145. Guy W. ECDEU assessment manual for psychopharmacology publication ADM 76-338. Washington, DC: U. S. Department of Health Education and Welfare, 1976:217-22.
146. McMahon LF, Jr., Wolfe RA, Huang S, Tedeschi P, Manning W, Jr., Edlund MJ. Racial and gender variation in use of diagnostic colonic procedures in the Michigan Medicare population. Med Care 1999;37:712-7.
147. Beskow LM, Burke W, Merz JF, Barr PA, Terry S, Penchaszadeh VB, Gostin LO, Gwinn M, Khoury MJ. Informed consent for population-based research involving genetics. JAMA 2001;286:2315-21.

148. Bremner JD, Vermetten E, Mazure CM. Development and preliminary psychometric properties of an instrument for the measurement of childhood trauma: The early trauma inventory. Depression and Anxiety 2000;12:1-12.
149 Fairburn CG, Beglin, SJ, The assessment of eating disorders: interview or self-report questionnaire? International Journal of Eating Disorders 1994; 16:363-370.
150 Elder KA, Grilo, CM, Masheb RM, Rothschild BS, Burke-Martindale CH, Brody ML. Comparison of two self-report instruments for assessing beinge eating in bariatric surgery candidates. Behavior Research and Therapy 2006; 44:545-560.
151 Talley NJ, Zinsmeister AR, Melton LJ 3rd. Gastrointestinal tract symptoms and self-reported abuse: a population-based study. Gastroenterology 1994; 4:1040-9
152 Chitkara DK, van Tilburg MAL, Blois-Martin N, Whitehead WE. Early life risk factors that contribute to irritable bowel syndrome in adults: a systematic review. American Journal of Gastroenterology 2008; 103:765-774.
153 Leserman J, Drossman DA. Relationship of abuse history to functional gastrointestinal disorders and symptoms: Some possible mediating mechanisms. Trauma Violence Abuse 2007;8;331

Appendix A
Interim Analysis Plans per request of DSMB

FDTT Interim Analysis Plan

At the request of the DSMB an interim analysis will be done after 200 participants have completed the study. This will entail computing summaries for the primary endpoint of "symptom relief", which was defined in the protocol as: patient reporting adequate symptom relief during at least half of the last 10 weeks of treatment. A 'closed report' for the DSMB will provide this summary overall, by each of the strata, and by treatment group.

A formal analysis will consist of fitting a logistic regression model with symptom relief (y vs. n) as the response and treatment group (amitriptyline, escitalopram, or placebo) as the primary predictor variable with the 8 strata (center, gender, Hospital Anxiety Depression Scale, dyspepsia subtype, gastric emptying, nutrient drink test satiety volume, BMI, and race) as covariates. Although the dynamic allocation randomization algorithm uses dichotomized versions of the strata, the continuous scale versions of the HAD scale, gastric emptying T ½ values, maximum tolerated volume from the nutrient drink test, and BMI will be used in this model. This will allow somewhat increased power to detect treatment group differences due to a more refined measurement of between-subject variability. A conservative alpha level (0.001) will be used to assess treatment group effects to keep the overall, experiment-wise type I error rate close to 0.05 at the end of the study.

Assuming that treatment effects are homogeneous across study centers, it was estimated that an overall pooled comparison of each active treatment vs. placebo (at the final analysis) would require the number of subjects per treatment group listed in the table below to achieve 80% power at a two-sided alpha level of 0.025 (i.e., adjusting for two pairwise tests, each active drug against placebo). We considered a 20% therapeutic gain or greater over placebo to be clinically significant. It was anticipated that the logistic regression model analysis incorporating potentially important covariates would provide similar or better power to detect comparable treatment group effects, assuming no substantial interactions with covariates or differential site effects. We assumed a 25% drop out rate in each arm. Many of these subjects will need to be considered as "treatment failures", i.e. no adequate symptom relief, but some may have sufficient time on treatment (e.g. 8 of the last 10 weeks with 5 or more weeks in which they've reported adequate relief) to define the primary endpoint. The chosen sample sizes (N=133 / treatment group) were selected to account for the resulting attenuation in response rates due to dropouts. For example, assuming the placebo response rate is 30%, the response rate on active drug is ≥ 50%, a 25% drop out rate in both groups, and response rates half the size in the dropouts (e.g. those that have sufficient data with the others failures; 15% on placebo and 25% on active drug), implies a response rate of 44% ((50+8)/133) on active drug and 26% ((30+5)/133) on placebo. To get 80% power to detect a difference of 44% vs. 26% (at alpha=0.025) requires approximately 133 subjects per group.

Placebo response rate	Active treatment response rate	N per group required	Placebo response rate	Active treatment response rate	N per group required
20%	40%	98	35%	55%	116
	45%	65		60%	75
	50%	46		65%	52
25%	45%	107	40%	60%	118
	50%	70		65%	75
	55%	49		70%	51
30%	50%	113	45%	65%	116
	55%	73		70%	73
	60%	51		75%	49

At the interim analysis there will be approximately 66 patients per treatment arm which would provide approximately 80% power to detect a difference in response rates (placebo vs. each active arm) of ≥ 35% (e.g. 20% on placebo vs. 55% on drug) at a two-sided alpha level of 0.001. The attenuation in response rates due to dropouts would likely result in somewhat smaller differences between treatment groups and thus adequate power for only somewhat larger differences.