Characteristics of the Co-Morbidity of Irritable Bowel Syndrome: A Secondary Analysis of Existing Twin Data

By
Mary K. Wojczynski, M.P.H.

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Approved by:

Advisor: Kari E. North, PhD
Reader: Patrick Sullivan, MD, FRANZCP
Reader: Charles Poole, ScD, MPH
Reader: Robert Sandler, MD, MPH
Reader: Betsy Sleath, PhD, RPh

ABSTRACT

Mary K. Wojczynski: Characteristics of the Co-Morbidity of Irritable Bowel Syndrome: A Secondary Analysis of Existing Twin Data

(Under the direction of Kari E. North, Ph.D.)

Irritable bowel syndrome (IBS) is a chronic disorder whose manifestations typically fluctuate over time. Prior epidemiologic studies estimate the one-year prevalence as 7-20%, depending on criteria used to define IBS. Individuals with IBS demonstrate a high co-occurrence with common functional somatic syndromes and psychiatric disorders; however, the majority of these associations derive from non-population-based studies. We examined associations between IBS risk factors and Rome II-defined IBS in a U.S. population-based twin registry. Data from 4,591 male and female twins were available for this analysis. Variables representing self-reported presence of IBS, major depressive disorder (MDD), chronic widespread pain (CWP), fatiguing illness (CFS-like illness), Medical Outcomes Study short form (SF-12) scores and other personal characteristics were obtained through questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were

calculated as measures of association between IBS risk factors and IBS. The prevalence of lifetime IBS was 4.7% (95% CI: 4.1, 5.4). Positive associations were observed between IBS and lifetime MDD (OR=2.0, 95% CI: 1.5, 2.7), lifetime CWP (OR=3.9, 95% CI: 2.7, 5.5), lifetime CFS-like illness (OR=4.7, 95% CI: 3.0, 7.3), and female sex (OR=2.0, 95% CI: 1.4, 2.8). Age, body size, and SF-12 scores demonstrated approximately null associations with IBS.

We further examined the overlap of individuals with IBS and MDD since both disorders suggest a familial tendency and demonstrate a higher than expected co-occurrence. Using the population-based Swedish Twin Registry, we examined the genetic and environmental architecture of the co-occurrence of IBS and MDD among 31,407 twins who contributed information on medical data and personal characteristics via phone interview. Both the case-control study and the co-twin control study design demonstrated an increased association between MDD and IBS (OR=2.7, 95% CI: 2.3, 3.2), and (OR=2.2, 95% CI, 1.5, 3.2), respectively. Thus, genetic and environmental factors did not confound the association between MDD and IBS; rather one of these disorders may predispose individuals to the other disorder. The positive associations observed between MDD and IBS suggest a possible hypothesis whereby one disorder is part of the causal disease mechanism of the other disorder, thereby leading to a high co-occurrence between MDD and IBS.

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LIST OF ABBREVIATIONS

5-HIAA	Hydroxyindoleacetic Acid
5-HT	Serotonin
5-HT$_{1A}$	Serotonin Type 1A Receptor
5-HT$_{2}$	Serotonin Type 2 Receptor
5-HT$_{2A}$	Serotonin Type 2A Receptor
5-HT$_{2B}$	Serotonin Type 2B Receptor
5-HT$_{3}$	Serotonin Type 3 Receptor
5-HT$_{4}$	Serotonin Type 4 Receptor
5-HT$_{6}$	Serotonin Type 6 Receptor
5-HTTLPR	Serotonin Transporter Long Polymorphic Repeat
ACE	Acetylcholine
ADIS-R	Anxiety Disorders Interview Schedule, Revised
AHP	Adult Health and Personality Survey
BDI	Beck Depression Inventory
BDQ	Bowel Disease Questionnaire
BIOMED1	European Collaborative Biomedical Research Project 1
BIOMED2	European Collaborative Biomedical Research Project 2
BMI	Body Mass Index
Bp	Base Pair
BSQ	Biliary Symptom Questionnaire

CCK(STR)	Cholescystokinin (Short Tandem Repeat)
CDC	US Center for Disease Control
CFS	Chronic Fatigue Syndrome
CI	Confidence Interval
CIDI	Composite International Diagnostic Interview
CLOCK	Circadian Locomotor Output Cycles Kaput
COMT	Catechol-O-Methyl Transferase
CRHR2	Corticotropin-Releasing Hormone Receptor 2
CSF	Cerebrospinal Fluid
CTLA4	Cytotoxic T Lymphocyte-associated 4
CWP	Chronic Widespread Pain
CYP2C9*3	Cytochrome P450 2C9
DAG	Directed Acyclic Graph
DAT1	Dopamine Transporter Gene
DDC	Dopa Decarboxylase
DID	Diagnostic Inventory for Depression
DIS	Diagnostic Interview Schedule
DNA	Deoxyribonucleic Acid
DRD2	Dopamine Receptor D2
DRD3	Dopamine Receptor D3
DRD4	Dopamine Receptor D4
DSM	Diagnostic and Statistical Manual

DSM-III	Diagnostic and Statistical Manual, 3rd edition
DSM-III-R	Diagnostic and Statistical Manual, 3rd edition, Revised
DSM-IV	Diagnostic and Statistical Manual, 4th edition
DZ	Dizygotic
ECPAD	European Collaborative Project on Affective Disorders
EMM	Effect Measure Modification
FBD	Functional Bowel Disorders
FDA	Food and Drug Administration
GABRA3	Gamma-Aminobutyric Acid Receptor, alpha-3
GEE	Generalized Estimating Equations
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GNAL	Guanine Nucleotide-binding Protein, alpha-activating activity polypeptide, olfactory type
GPRD	General Practice Research Database
GSK	GlaxoSmithKline
HR	Hazard Ratio
HTR1B	Serotonin Transporter Type 1B Receptor
HTR2A	Serotonin Transporter Type 2A Receptor
HTR2C	Serotonin Transporter Type 2C Receptor
HTR5A	Serotonin Transporter Type 5A Receptor
HTR6	Serotonin Transporter Type 6 Receptor

I/D	Insertion/Deletion
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICD	International Classification of Disease
ICD-10	International Classification of Disease, 10th edition
ICD-8	International Classification of Diseases, 8th edition
ICR	Interaction Contrast Ratio
IDD	Inventory to Diagnose Depression
IDS	Inventory for Depressive Symptomatology
IL10	Interleukin 10
IRB	Institutional Review Board
LOD	Log Odds
MAO	Monoamine Oxidase
MAOA	Monoamine Oxidase A
MATR	Mid-Atlantic Twin Registry
MD	Doctor of Medicine
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
MDL	Major Depression, Lifetime
MZ	Monozygotic
NCS-R	National Comorbidity Study—Replication
NE	Norepinephrine

NET	Norepinephrine Transporter
NS	Not Significant
OPCRIT	Operational Criteria Checklist
OR	Odds Ratio
PhD	Doctor of Philosophy
PLA2	Phospholipase A2
PSE	Present State Exam
RDC	Research Diagnostic Criteria
RE-MDD	Recurrent, Early-onset Major Depressive Disorder
RFLP	Restriction Fragment Length Polymorphism
RR	Relative Risk
SADS	Schedule of Affective Disorders and Schizophrenia
SADS-L	Schedule of Affective Disorders and Schizophrenia, Lifetime version
SALT	Screening Across the Lifespan Twin Study
SAS	Statistical Analysis Software
sBSQ	Biliary Symptom Questionnaire, shortened version
SCID	Structured Clinical Interview for DSM-IV
SCID-II	Structured Clinical Interview for DSM-III, Axis II Disorders
SCID-I-NP	Structured Clinical Interview for DSM-III, Axis I Disorders, non-patient
SDI	Short Depression Interview

SE	Standard Error
SEM	Structured Equation Modeling
SERT	Serotonin Transporter
SES	Socio-economic Status
SF-12	Medical Outcomes Study 12-Item Short Form Health Survey
SF-12v2	Medical Outcomes Study 12-item Short Form Health Survey, version2
SF-36	Medical Outcomes Study 36-item Short Form Health Survey
SIBO	Small Intestinal Bacterial Overgrowth
SLC6A4	Serotonin Transporter Protein
SNP	Single Nucleotide Polymorphism
SPIKE	Structured Psychopathological Interview and Rating of the Social Consequences of Psychic Disturbances for Epidemiology
SSRI	Selective Serotonin Reuptake Inhibitor
SSTRP	Single Sequence Tandem Repeat Polymorphisms
STR	Swedish Twin Registry
TGFB1	Transforming Growth Factor β1
TH	Tyrosine Hydroxylase
TPH1	Tryptophan Hydroxylase
TPH2	Tryptophan Hydroxylase 2
UK	United Kingdom
US	United States

VNTR	Variable Number Tandem Repeats
WFS	Wolfram Syndrome
WFS1	Wolfram Syndrome Gene

1 REVIEW OF THE LITERATURE

1.1 SUMMARY

This dissertation examines Rome II defined irritable bowel syndrome (1) and other disorders associated with IBS. I describe the prevalence of Rome II defined IBS in a population-based sample, as well as further elucidating the covariates associated with Rome II IBS. Additionally, because major depressive disorder (MDD) has a tendency to be associated with IBS, the lifetime co-occurrence of these two common disorders, IBS and MDD, is further analyzed using twin data to investigate the possible genetic and environmental effects involved in the co-occurrence of these two disorders.

This chapter briefly reviews the background of IBS and then MDD. The review includes the definition of IBS, the epidemiology of IBS, the pathophysiology of IBS, and the genetic factors involved in IBS. The review of MDD includes the definition of MDD, the epidemiology of MDD, the pathophysiology of MDD, and the genetic factors involved in MDD. Finally, the review of the co-occurrence of IBS and MDD includes the epidemiology of the co-occurrence of IBS and MDD as well as the proposed mechanism for their co-occurrence.

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 Definition of Irritable Bowel Syndrome

A. Clinical Definition

Irritable bowel syndrome is a chronic disorder of the gastrointestinal (GI) tract. It is characterized by exacerbations and remissions of abdominal pain and/or discomfort that leads to alterations in bowel habits (2-6). IBS is a syndrome that falls under the classification of a functional gastrointestinal disorder. The etiology of IBS is not known. The abdominal pain and/or discomfort that individuals with IBS have is unexplained by other organic disease that is detectable with currently available diagnostic methods (3, 5). There is no diagnostic test nor biological indicator for IBS (4, 7). Rather the diagnosis is based on symptom criteria and exclusion of structural disease (2, 4).

B. Consensus Criteria

There are three similar, yet distinct sets of possible criteria to diagnose IBS. These are the Manning criteria (1978), the Rome I criteria (1990), and the Rome II criteria (1999) (5), presented in Table 1.1. Clinically, the Rome criteria (either I or II) are the most widely accepted research criteria for IBS. There are also red flags to the diagnosis, presented in Table 1.2. If any red flags are present in the patient, the physician should look for a diagnosis alternate to IBS (8).

C. Disease Subgroups

There are two distinct subgroups of individuals who have IBS, those who visit a physician for their symptoms and those who do not have such a physician visit (3, 4). In a large percentage (40-60%) of those who seek medical advice or treatment for IBS, there is a close association of psychological distress or affective disorders with somatic symptoms (2).

Only 10-30% of individuals with IBS seek health care for their symptoms (8). IBS sufferers constitute 20%-50% of referrals to GI clinics (4, 9). Health-care seeking individuals with IBS are more likely to be female, have a lower socioeconomic status (SES), have greater levels of psychological morbidity (including anxiety and depression), and experience a reduced quality of life (5, 8). Additionally consultation with a health care professional has been correlated with perceived seriousness of symptoms (10).

To be diagnosed with IBS, two main conditions must be met: 1) no diagnostic organic disease can be detected and 2) the diagnostic criteria (Rome I or II) need to be fulfilled in order to have confidence in a positive IBS diagnosis (4, 5). Although this is the common practice in diagnosing IBS, and the consensus opinion of expert gastroenterologists, it will continue to be controversial until the pathophysiology of IBS is better understood (11).

Once a diagnosis of IBS is obtained, it is further classified as either diarrhea-predominant, constipation-predominant, or pain/discomfort predominant IBS (3). These designations help with the therapeutic approach employed for each patient, since therapy is directed towards alleviating the worst symptoms (4).

D. Current Treatment

Currently, there is no cure for IBS. Treatment is aimed solely at alleviating symptoms and reducing disease related distress. A good patient-physician relationship is crucial for successful treatment of IBS symptoms. A large percentage of patients (40-70%) will have symptom improvement with placebo alone (4). Drug treatment should be targeted at alleviating the most bothersome symptoms (4, 12). Current treatments based on symptomatology are: fiber for constipation; loperamide for diarrhea; smooth muscle relaxants for pain; psychotropic agents for treating depression, diarrhea, and pain; and psychological treatments (12).

Newer therapies that have been introduced are 5-hydroxytryptamine$_3$ ($5HT_3$) receptor antagonists and 5-hydroxytryptamine$_4$ ($5HT_4$) receptor agonists and antagonists (12).The $5HT_4$ agonist tegaserod has been shown to benefit those with constipation predominant IBS (7). Significant symptom improvement has been shown with the use of specific antagonists of $5HT_3$ receptors, which act peripherally at the enteric nervous system (13).

Alosetron, a $5HT_3$ antagonist has been shown to be effective in relieving pain and diarrhea in women with diarrhea-predominant IBS (14). One of the adverse effects of alosetron is that it has been shown to cause constipation in a dose-dependent manner (15). Additionally, a more serious adverse event, ischemic colitis, was reported after administration of this drug (15, 16). The combination of these two serious adverse events led to the voluntary removal of alosetron from the market by its manufacturer, GlaxoSmithKline (GSK), in November 2000 (15-17), only seven months after its initial approval (16). Following this drug withdrawal, patient advocate groups sent letters to both the Food and Drug Administration (FDA) and GSK, stating how alosetron lessened IBS symptoms and improved their quality of life (16, 17). The FDA and GSK met and created a risk management plan for alosetron that was submitted with a supplemental new drug application, approved by the FDA on June 7, 2002 (15-17). Based on the risk management plan and the subsequent re-approval, alosetron is only available in the United States (US) to a select group of patients, and only from doctors enrolled in the alosetron prescribing program (17). The only approved indication for alosetron is for women with severe diarrhea-predominant IBS who have symptoms for at least 6 months and who have failed conventional therapy (16, 17).

1.2.2 Epidemiology of Irritable Bowel Syndrome

A. Public Health Burden

IBS poses a substantial public health burden. Many studies estimate that 9-22% of US adults have a lifetime history of IBS (8). IBS is a common condition and can co-exist with organic disease (3). Through the use of the International Classification of Disease (ICD) coding system, it is estimated that 15 million individuals in the United States are suffering from IBS (18).

B. Public Health Significance

As these numbers indicate, IBS is a substantial burden to the health care system. It is estimated that IBS was responsible for 3.7 million physician office visits in 1998 (18). In a community-based study in Minnesota, individuals with IBS incurred a substantial excess of physician charges when compared to control subjects of similar age and sex (9). In terms of days lost from work, sufferers of IBS report missing more days of work due to their symptoms than any other disease/condition except the common cold (19, 20). The Minnesota study only included direct medical costs (9), thus if treatment and prescription costs are included, as well as days lost from work, the financial burden of IBS to the community would be even larger. For the year 1998, a conservative estimate of the total health care costs (direct and indirect) in the United States is $1.5 billion (18). Health care utilization and

costs are not just an issue in the US. In China, the diagnosis of IBS was significantly correlated with increased health care utilization (20).

C. IBS Prevalence Estimates

Most papers cite the one-year or current prevalence of IBS to be anywhere from 9% to 22% in the US as determined by community-based studies (5, 6, 8), with the majority having a prevalence between 10-15% (5, 9, 21). While this demonstrates that a substantial number of individuals have the disease, it also demonstrates the variability in its diagnostic definition (22). To obtain a more realistic estimate of the population prevalence of IBS, it is necessary to examine the prevalence for each specific set of criteria, the Manning criteria, Rome I criteria, and Rome II criteria (23-26). The Manning criteria are the most inclusive, and therefore give the highest range in IBS prevalence. In contrast, the Rome II criteria appear to be the most restrictive, with the lowest IBS prevalence. The Rome I criteria are between the previously stated criteria. As a research definition of IBS, the Rome II criteria are increasingly becoming the standard as the criteria are more specific (27). Table 1.3 summarizes the published literature on the population prevalence of IBS since 1995 (5, 9, 20, 23-25, 28-30).

Additionally, since women usually have IBS at a rate of 2:1 compared to men (7), some studies have obtained estimates stratified by gender. In these studies, the prevalence range for women, employing Rome II criteria, is

3.8-5.4% (26, 31). When using Rome I criteria for women, the prevalence range is 10.7-18.1% (21, 25). Among men, there is only one study with adequate methods that used the Rome II criteria, obtaining a prevalence of 3.6% (31), and by Rome I criteria the prevalence range among men is from 5.8% to 8.5% (21, 25). Thus it appears as though the 2:1 female predominance is maintained for the Manning and Rome I IBS definitions, but may be slightly less when using the Rome II definition of IBS.

Studies have also demonstrated a difference based on geographic location. Population-based studies with adequate methods and adequate sample size have been performed in Australia/New Zealand (5, 28, 29), and a recent one performed in 8 European countries (10). Additional countries which had adequate studies performed were Spain (23), United States (9, 24), Hong Kong (20, 30), Canada (21), and Bangladesh (25). The prevalence ranges for these studies are presented in Table 1.4. Studies that used the Manning criteria in Australia/New Zealand, Spain, and the US obtained similar prevalences, however the study of 8 European countries had a lower prevalence. Rome I prevalence ranges varied from 4-12% across all geographic locations, while Rome II prevalence ranges were similar in Australia/New Zealand, 8 European countries, Hong Kong, and Spain, but the study performed in Canada was a distinct outlier. This can be explained by the use of 'modified' Rome II criteria (21).

Based on this simple review of the literature on the population prevalence of IBS, it is clear that the definition used to obtain the prevalence estimate needs to be reported along with the prevalence. Using this logic and the accompanying tables, it appears that the cited prevalence range of 9-22% has been derived using mainly the Manning criteria, and possibly the Rome I criteria. The prevalence estimate of 10-15% is more than likely applicable to the diagnosis of IBS using the Rome I criteria. Finally, since the majority of studies published in 2002 and later used the Rome II criteria, a population prevalence range of 4-7% by the Rome II criteria is probably the most accurate estimate based on our current knowledge.

D. IBS Risk Factors

***Age*.** Although age is not significantly associated with a diagnosis of IBS (5), half of IBS sufferers have symptoms that occur by age 35 (4). Additionally, patients younger than 45 years of age were more likely to be diagnosed with IBS than were those older than 45 years (7). Based on the systematic review by Saito et al., the prevalence of IBS varies minimally with age (32). Thus while age is important as a clinical descriptor of IBS, it is not a risk factor for IBS since there is no increase in risk associated with different age groups.

***Gender*.** When examining gender as a risk factor for IBS, most studies have demonstrated a 2:1 female predominance of IBS in North American

population-based studies (7). This is also substantiated by examining the gender-specific prevalence rates reported in the studies mentioned above (7, 21, 25, 26, 31), which are representative of different areas of the world.

***Body mass index*.** In studies examining body mass index (BMI) as a risk factor for IBS, results are conflicting. In a US community-based study by Locke et al., no association was identified between IBS and BMI (33). Additionally, in a study of Swedish IBS patients by Simren et al., BMI was similar in patients and controls, and thus not associated with IBS (34). In a clinic based study in Taipei, China, BMI was not significantly different among those with constipation-predominant IBS, diarrhea-predominant IBS, or controls; however BMI did have similar positive correlations with gastrointestinal transit times (35). Despite these negative findings, a recent community-based study in Croatia demonstrated that an increase in BMI of 5 kg/m^2 increased the risk of IBS 36% (36). Thus while evidence is leading towards BMI not being a risk factor for IBS, there is still controversy and more research needs to be done.

***Concurrent medical conditions*.** All definitions of IBS (Manning, Rome I, or Rome II) have been shown to occur concurrently with many other medical conditions. In a large European study, 21% of IBS sufferers also had gastroesophageal reflux disease (versus 7% among non-IBS sufferers), 13% had peptic ulcer (versus 6% among those without IBS), 13% had dyspepsia

(versus 4% among those without IBS), 25% had depression (versus 9% among those without IBS), and 13% had asthma (versus 7% among those without IBS) (10).

***Psychological disorders*.** Psychological disorders are common co-morbid conditions in most IBS patients who actively seek subspecialist medical care (7). Blanchard et al. have shown through the use of a structured interview, the Anxiety Disorders Interview Schedule—Revised (ADIS-R), that IBS patients have greater psychopathology and are more anxious and depressed than either inflammatory bowel disease (IBD) patients or healthy controls (37). In a study of 74 IBS patients seen at an outpatient clinic, the depression score on the Zung Depression Self-Rating Scale was significantly higher in patients with IBS compared to controls (38).

In a study of psychiatric patients, the point prevalence of IBS was 29% in patients with major depression and 37% in generalized anxiety disorder patients as compared to 11% in control participants (39). Furthermore, of patients seen in specialist clinics, IBS patients showed increased psychological morbidity (40-44). Patients with IBS were shown to have higher anxiety and depression scores, as assessed using the hospital anxiety and depression scale, when compared to patients with no IBS (20). More discussion of the epidemiology of IBS and MDD co-occurrence will be discussed on page 53.

***Fibromyalgia*.** Fibromyalgia also co-occurs with IBS at a substantial frequency. Ranges of this co-occurrence are 32-66% (Rome criteria for IBS) (45-48). Additionally, fibromyalgia and IBS share some epidemiologic features, such as a predominance of females (46, 48, 49) and associations with sleep disturbances, depression, anxiety (46), and muscle and abdominal pain (50), supporting a common pathophysiologic mechanism (46, 48, 51). In addition, fibromyalgia patients have a clear dysregulation of their autonomic nervous system (pathology unknown) and also exhibit low serum levels of the serotonin precursor L-tryptophan (51), two possible etiologic hypotheses for IBS. However, their diagnostic criteria overlap, which may be responsible for some of the demonstrated co-occurrence (46, 49, 52). Additionally, a difference between these two disorders has been demonstrated. IBS patients demonstrate visceral hypersensitivity, as measured by rectal balloon distention, and this characteristic is not shared with fibromyalgia patients, both those with and without co-occurring IBS (46).

Hudson et al. studied 33 women with fibromyalgia and found that 39% had a current diagnosis of IBS, and 52% had a lifetime diagnosis of IBS (53). Additionally, of those with IBS and fibromyalgia, 47% had IBS for a year or more before the onset of fibromyalgia, 12% had IBS and fibromyalgia onset during the same year, and 41% had IBS onset a year or more after the diagnosis of fibromyalgia (53). Thus these two disorders are associated over a patient's lifecourse.

Chronic fatigue syndrome. Chronic fatigue syndrome (CFS) is also associated with IBS (50, 52, 54). CFS patients were more likely than controls to demonstrate symptoms of IBS according to the Manning criteria (50). Symptoms common to both disorders include muscle and abdominal pain (52), sleep, and concentration difficulties (50). The rate of IBS among CFS patients ranges from 50-92% (50, 54). Specifically, one study found that 73% of chronically fatigued adults met the Manning criteria over a 1-year retrospective time period (55). These rates, while determined with differing IBS criteria, are well above the estimated population prevalence of IBS (54). Again, the etiology of CFS is unknown, and whether the apparent overlap results from shared underlying mechanisms or coexisting psychiatric disorders remains controversial (54). In a twin study, fatigued twins were 4-10 times more likely to be diagnosed with IBS compared to their unaffected co-twins, regardless of the definition of IBS (54).

Asthma. Asthma has also demonstrated an association with IBS (56-59). The study by Roussos et al. included two control groups: healthy controls and controls with other pulmonary disorders (56). Results demonstrated that regardless of the control group, the IBS prevalence was significantly higher in asthmatics (56). Similarly, Huerta et al. examined a cohort of asthmatics in the General Practice Research Database (GPRD) and also found a slight increased risk of IBS among asthmatics compared to the United Kingdom (UK) general population (58). Yazar et al. examined cases

of IBS, using only one control group, and found that IBS patients had a higher prevalence of asthma than controls (57). The study by Kennedy et al. examined bronchial hyper-responsiveness and demonstrated an independent association between IBS and bronchial hyper-responsiveness (59). Thus it is apparent that IBS and asthma have a tendency to co-occur.

***Family history*.** An increased likelihood of bowel symptoms among biologic relatives of IBS cases has been demonstrated (60). Using logistic regression in a twin analysis, it has been shown that having a mother with IBS and having a father with IBS are independent predictors of irritable bowel status ($p<0.001$), and both are stronger predictors than having a twin with IBS (61). Further discussion of the familial clustering of IBS will be covered in greater detail on page 19.

E. Quality of Life

Patients with IBS report a reduced quality of life, as measured using the Medical Outcomes Study 36-item short form health survey (SF-36) (62-65) or the shorter Medical Outcomes Study 12-item short form health survey, version 2 (SF-12v2) (66). There is also a tendency for a lower SF-36 score based on severity of IBS, with the lowest scores for patients with the most severe IBS (62, 67). Additionally, this relationship was also observed when compared with gastroesophageal reflux disease controls, asthma controls, or migraine controls (65). In a population-based, nested, case-control study, the

SF-36 was used to initially demonstrate that health-related quality of life is impaired in community subjects, consisting of both health-care consulters and non-consulters, with IBS (68). After further analyses in this study, it was demonstrated that the apparent association could be explained by the Symptom Checklist-90 somatization score (68). Taken together, these studies demonstrate that quality of life is impaired among individuals with IBS.

1.2.3 Pathophysiology of Irritable Bowel Syndrome

A. Possible Etiologies

Since the etiology of IBS is largely unknown, there are several mechanisms proposed to produce the symptoms of IBS. These include, but are not limited to, altered motility, visceral hypersensitivity, abnormal brain-gut interaction, autonomic dysfunction, immune activation (69), intestinal luminal irritants, psychological distress and psychiatric disease, post-infectious or post-inflammatory phenomenon, abnormal motor function (gastrointestinal motility), and abnormal visceral perception (32). Additionally, investigators have postulated that disturbances in intestinal motility result in symptoms of abdominal pain, bloating, and disturbed defecation (7). In 92% of IBS patients, regardless of their IBS type, there is a common symptom of bloating, indicating that there may be a unifying theory of IBS that is not yet known (69).

A dysfunction of the brain-gut axis, linking the central nervous system, autonomic nervous system, enteric nervous system, and related humoral and immunological processes may be involved in the disturbances of motility and visceral sensitivity associated with IBS (70, 71). The visceral sensitivity associated with IBS means that patients with IBS sense pain or discomfort with less distension of the colon than do control patients (7). Burr et al. have shown that severe pain intensity and pain that is not postprandial are both associated with significantly lower average vagal activity and higher average sympathetic nervous system/parasympathetic nervous system balance (70). The dysfunction of the brain-gut axis may trigger a number of inappropriate reflexes which alter gastrointestinal motility, secretion and absorption, thus causing the wide variety of symptoms often associated with IBS (71).

The small intestinal bacterial overgrowth (SIBO) theory comes from the observation that in normal, non-IBS individuals, the bacteria that ferments undigested food are located in the colon; however it has been demonstrated that in IBS patients, due to low food motility, undigested food does not proceed to the colon. Thus the colonic bacteria migrates to the undigested food in the distal small intestine, thereby colonizing the small intestine, producing gas and fermentation of undigested food (69). Recently the SIBO theory of IBS (69) explains postprandial bloating and distension, altered motility (1), visceral hypersensitivity (72), abnormal brain-gut interaction (73), autonomic dysfunction (74), and immune activation (69). Postprandial

bloating is common in most IBS sufferers, yet the prior IBS pathophysiologic theories of abnormal motility, visceral hypersensitivity, altered brain-gut interaction, autonomic dysfunction, and immune activation do not account for this predominant symptom (69). Support for the SIBO theory of IBS comes from the reduction of both gastrointestinal and extraintestinal symptoms when eradication of SIBO is achieved (69).

B. Serotonin Involvement in IBS Etiology

Serotonin (5-HT), a neurotransmitter, may be involved in irritable bowel syndrome (8). Of the serotonin in the body, it is estimated that 95% is present and functioning in the gastrointestinal tract (75). Of the serotonin found in the GI tract, it has been estimated that 90% is in enterochromaffin cells and 10% is found in enteric neurons (75). Serotonin is released from the bowel when enteric nerves are stimulated (75). Serotonin modulates sensorimotor functions in the digestive tract (76).

Seven subclasses of 5-HT receptors are defined. These receptors are differentiated on the basis of structure, molecular mechanism, and function (76). The type 3 receptor ($5\text{-}HT_3$) is known to partially mediate the postprandial colonic motor response (77) that is often associated with cramping, urgency, and diarrhea in patients with IBS (78, 79). The type 2B receptor ($5\text{-}HT_{2B}$) has been shown to mediate the excitatory effects of 5-HT in the human colon (80). The $5\text{-}HT_{2B}$ receptors have been localized on both the

colon smooth muscle and on the nerves of the myenteric plexus. Thus, 5-HT may be exerting its effects on smooth muscle directly, or in combination with increasing the release of excitatory neurotransmitters (80).

Serotonin is a known modulator of sensorimotor functioning in the digestive tract, as is demonstrated by the response to specific serotonin receptor agonist and antagonist drugs. It is thought that 5-HT may stimulate intestinal secretion and peristalsis in addition to the usual visceral pain receptors via 5-HT_3 and 5-HT_4 pathways (8). Two drugs, alosetron and tegaserod, are used for the treatment of IBS at target these receptors. Alosetron is a 5-HT_3 receptor antagonist that is more effective than placebo at relieving global IBS symptoms in female IBS patients with diarrhea (7, 15). Antagonists of 5-HT_3 receptors act peripherally at the enteric nervous system to decrease motility and visceral sensitivity (15) and significantly improves IBS symptoms (13). Alosetron slows colonic transit and decreases discomfort during distension of the colon (7). However alosetron has some serious side-effects associated with its usage, as previously discussed.

Tegaserod, a 5-HT_4 receptor agonist, was more effective than placebo in randomized clinical trials at relieving global IBS symptoms in female IBS patients with constipation (7). Tegaserod stimulates the peristaltic reflex, increases intestinal and colonic transit, reduces the firing rate of rectal afferent nerves, and reduces visceral sensitivity (7).

Reuptake of serotonin in the intestine is under control of the serotonin transporter protein (*SLC6A4*) (16, 81). Polymorphisms in *SLC6A4* may affect responses to serotonergic medications (15) by affecting the efficiency of the serotonin transporter in removing 5-HT from the synapse. The study by Camilleri, et al., showed that the homogeneous long polymorphism (L/L) of the *SLC6A4* promoter in IBS patients was associated with a longer transit time through the colon in response to alosetron compared to heterozygous patients (76). Thus there may be a genetic susceptibility to IBS treatment response, and in this case specifically to alosetron (16, 76).

1.2.4 Role of Genetics in Irritable Bowel Syndrome Etiology

A. Family Studies in IBS

Substantial evidence for the clustering of IBS in families is documented in the literature and presented in Table 1.5. These studies comprised a mix of population and clinical cohorts, with the majority of recent studies examining population-based samples. Methodologically, the Locke (60) and Kalantar (82) studies used formal family methods whereas the others demonstrated a family history of IBS using family history as a covariate. Additionally, only the latter studies presented in the table used research criteria specific for IBS.

In the Locke et al. (60) study, applying the Rome I criteria for IBS, the odds of IBS were twice as great (odds ratio (OR) 2.3, 95% confidence interval

(CI) 1.3-3.9) in first degree relatives with abdominal symptoms in comparison to first degree relatives without abdominal symptoms, adjusted for age, sex, and psychosomatic symptom checklist score (60). Evidence for the familial aggregation of IBS is additionally supported by Kalantar et al., where among those with a positive family history of IBS, the odds of IBS were twice as great for those with persistent or fluctuating IBS versus those with no IBS (83). The other family study completed by Kalantar et al. also demonstrated a familial component to IBS (82) with the odds of IBS nearly three times higher among those with a first-degree relative with IBS than with an affected in-law (OR 2.7, 95% CI 1.19-6.25), adjusted for age and sex (82). When this model was further adjusted for somatization score, the OR was attenuated and no longer significant. Thus while these results support a familial component to IBS, it is possible that the aggregation may be due to another disorder (82, 84). As these studies demonstrate, there is substantial evidence to support a familial component to IBS.

***Limitations*.** There are limitations to these studies that may affect the validity of their conclusions. One of the limitations is the possibility of reporting bias, as individuals with IBS may preferentially remember relatives that had abdominal problems. Additionally, the assessment of family history may not be very rigorous, as demonstrated in the Locke et al. (60) study. The question asked broadly about relatives with abdominal symptoms, used no time frame for those symptoms, and cited only a fair reliability (κ=0.48) for

this question (60). Also, the definition of IBS used in these studies was either Manning, Rome I, or abdominal pain, and the results may not reflect the prevalences that would be observed using Rome II criteria, which are more restrictive than the other criteria. It has also been suggested that the demonstrated aggregation may be due to another disorder, possibly somatization (82). Finally, the demonstrated aggregation could be attributed to a common childhood environment or even to a possible gene-environment interaction (82), neither of which are possible to ascertain using family study methodologies.

B. Adoption Studies

There are no adoption studies for IBS. If adoption studies were performed for IBS, the results would help to distinguish genetic effects from shared environmental effects on the etiology of IBS.

C. Twin Studies

***General Method*.** Twin studies often examine the relative importance of genetic and environmental influences for behavioral characteristics and diseases. The use of twin studies in research is attractive because it uses the natural biological phenomenon of twinning in order to differentiate environmental and genetic factors in disease etiology. Using principles of biology and genetics, the twin phenomenon implies that monozygotic (MZ) twins are, for most purposes, genetically identical, whereas dizygotic (DZ)

twins share the same amount of genes as would two normal siblings. That is dizygotic twins share on average half of their genes, identical by descent, and monozygotic twins share all their genes, thus being genetically identical. To use this phenomenon of twinning in research, one compares the similarity of monozygotic twin pairs for a trait or a disorder with the similarity of dizygotic twin pairs, and thereby can detect environmental and genetic effects, and most importantly, has the ability to quantify the magnitude of these effects (85-87). This is especially attractive when examining co-occurring disorders because twin studies control, to a substantial extent, genetic and environmental factors, thus helping to elucidate the complex interactions of biological, psychological, and environmental factors involved in co-morbid conditions (54, 87).

Classic twin modeling partitions the variation in liability to a disorder by means of decomposition of variance (88). This is done using structured equation modeling (SEM), as performed by various software programs such as Mx (89). This analysis uses path diagrams and matrix algebra to determine the decomposition of variances and covariances, and in doing so is able to determine what percent of the demonstrated association is due to additive genetic effects (a^2) (also known as heritability), common environmental effects (c^2), and unique environmental effects (e^2) (87, 88), which also encapsulates measurement error. This analysis is heavily

dependent on knowing the pairwise zygosity of the twins as well as their affection status.

Results of Twin Studies in IBS. Four twin studies examining the genetic liability to IBS are described in Table 1.6. All three twin studies were population-based samples of twin pairs. Methodologically all but the third study employed classic twin methods, examining monozygotic (MZ) and dizygotic (DZ) concordance rates, whereas the third study employed a co-twin control design. A drawback of these studies is the lack of use of a consensus definition of IBS, either Manning, Rome I, or Rome II. The Svedberg et al. (90) study employed a diagnostic algorithm to define IBS that is similar to the Rome criteria, however use of a consensus definition would help comparability and consistency of IBS definition across different studies.

The study by Morris-Yates et al. demonstrated a higher concordance of IBS in MZ twins than in DZ twins (33.3% versus 13.3%, respectively) (91). Similar results were reported in the twin study by Levy et al., where the MZ twin concordance was 17.2% and DZ twin concordance was 8.4% for IBS (61). While the concordances are considerably higher in identical (MZ) twins, there is not a perfect concordance of 100%, suggesting moderate to high environmental influences (86). Morris-Yates et al. alluded to this in their analysis where 58% of the difference in liability to functional bowel disorders (FBD) was attributed to genetic control (91), and not 100% as perfect MZ

concordance would demonstrate. One problem with this study is that the assessed phenotype of FBD includes a mix of individuals with IBS, functional abdominal bloating, functional constipation, functional diarrhea, and functional abdominal pain. Thus the results may not be true of the individual disorders that fall under the realm of FBD. Together, these results are suggestive of both genetic and environmental contributions to the etiology of IBS.

In contrast, the classic twin study by Mohammed et al. (92) demonstrated similar IBS concordance rates between MZ and DZ twin pairs (28% and 27%, respectively). Additionally, the best-fitting classic twin model did not contain a variable for heritability, but only variables for common and unique environmental influences. Thus this study demonstrated that genetic factors have little or no influence over IBS. The main difference between this twin study and the previous studies was the use of the most recent, more restrictive Rome II IBS criteria.

The third twin study by Svedberg et al. performed a case-control and co-twin analysis. The case-control analysis demonstrated associations between IBS and eating disorders (OR 2.4; 95% CI 1.1-5.1), urological problems (OR 3.3; 95% CI 1.3-8.2), poor self-rated health (OR 1.8; 95% CI 1.0-3.2), eating allergies (OR 9.0; 95% CI 1.4-60.1), and rheumatoid arthritis (OR 3.2; 95% CI 1.1-9.4) (90). The co-twin analysis examined 58 disease discordant MZ twin pairs and only the association between urologic problems

and IBS remained (OR 4.0; 95% CI 1.0-16.8), suggesting a common etiology not attributable to genetic effects (90). The associations between IBS and eating disorders, eating allergies, and rheumatoid arthritis decreased in the co-twin analysis, suggesting genetic as well as family environmental effects on these associations (90).

In summary, three of these twin studies are supportive of both genetic and environmental components to IBS; however several problems were noted. First, a consensus definition of IBS was not employed, and one study used the broader term of functional bowel disorders and applied the results to IBS. Thus a better defined phenotype may add to this existing knowledge. Another criticism of twin studies is the inability to separate similarity due to common environment (from conception onward) from similarity due to genetic influences (86). Despite these limitations, there were strengths. These studies all used population-based twin registries and twin study methodologies. Taking the results of twin and family studies together, there is accumulating evidence suggestive of genetic and environmental contributions to IBS.

D. Linkage Studies

***General Method*.** Linkage analysis is used to determine the chromosomal location of genes that affect a given disorder. It is based in classical genetics and relies on the violation of Mendel's second law; the law

of independent assortment. Independent assortment dictates that loci on a chromosome are able to separate from each other during meiosis and thus be transmitted to successive generations independently. The violation of independent assortment means that when two loci on the same chromosome are in close proximity, the alleles at these loci tend to sort together ("cosegregate") within a pedigree (93), thus violating the independence between many loci. Additionally, the degree of cosegregation is loosely dependent upon the genetic distance between the two loci (93). Thus we assume that the genotype at the marker locus represents the genotype at the surrounding sequence, which may harbor the functional variant associated with disease etiology. Significant evidence for linkage to a phenotypic trait is determined either by consistent transmission of a phenotype or disease from parent to offspring, proportion of alleles shared between affected and unaffected siblings, or the relationship between allele sharing and means or differences in phenotypic values for pairs of relatives. Traditional linkage analysis is performed using family pedigrees and thus any demonstrated linkage is family specific and needs to be replicated in different pedigrees, unless there is considerable linkage disequilibrium (93, 94).

***Results in IBS*.** To date, no linkage studies of IBS have been performed.

E. Candidate Gene Studies

***General Methods*.** Candidate gene studies, or association studies, are used to identify possible causal variants in candidate genes. A variety of samples, including case-control, case only, and family data, are used to find disease pre-disposing alleles (94). In population based case-control association studies, the most commonly implemented design, the statistical comparison is the marker allele frequency between cases (diseased or affected) and controls (non-diseased or non-affected) (86). This methodology thus identifies alleles or combinations of alleles that occur more often than predicted by chance among individuals with a particular phenotype (cases) than among non-cases (86, 93). Of considerable importance in the design of association studies is control selection, as controls should be chosen to represent the population from which cases are derived (93), and thus represent the same underlying population. This is important because allelic frequencies for deoxyribonucleic acid (DNA) markers have been shown to vary among different ethnic groups (86, 93). Additionally, the controls are used to estimate the prevalence of the marker alleles in the source population (93), and if the case and control populations are not from the same source population, the prevalence of the marker alleles in cases is not being compared to the correct prevalence of marker alleles in controls, thus the possibility of incorrect association. In addition to needing special care for control selection, association studies are subject to the same biases as

traditional case-control studies (low power, selection bias, misclassification, and confounding) (93).

Until such time that full genome association studies are affordable for non-industry investigation, the majority of population-based research will likely use a candidate gene approach. The selection of candidate genes should be made based on a biologically plausible hypothesis; such that the gene product is involved in the phenotype of interest. Information on the function of candidate genes and the identification of important polymorphisms and validated single nucleotide polymorphisms (SNPs) within these genes can be obtained at various on-line resources.

***Results in IBS*.** Table 1.7 summarizes the published candidate gene studies for IBS. These studies were performed in clinic-based samples, and suffer from issues of health-care seeking influence, poor marker coverage of the gene, and low power. To date, the serotonin and transforming growth factor β_1 (*TGFB1*) polymorphisms that were investigated were not successful in demonstrating an association with IBS. The association between the polymorphism in Interleukin 10 and IBS, while not statistically significant, is of further interest because the study was slightly underpowered to detect such a small result. Also of further interest is the polymorphism in the study by Camilleri, et al., because the L/L homozygote demonstrated delayed colonic emptying (76), regardless of the fact that the study was underpowered.

Despite being the most severely underpowered study, the results of Pata et al. suggested that the S/S and L/S genotypes of the *SLC6A4* gene-linked polymorphic region [*5-HTTLPR*] may predispose individuals to certain subtypes of IBS (81). Thus while none of these studies have shown genetic polymorphisms associated with IBS, there are other polymorphisms in these genes that can be investigated, as well as performing studies on these same polymorphisms using larger sample sizes in order to obtain adequate power to detect a smaller effect.

1.2.5 Definition of Major Depressive Disorder

A. Clinical Definition

Major depressive disorder (MDD) is a medical illness that is characterized by abnormalities of affect and mood, appetite, sleep disturbances, inappropriate feelings of guilt and worthlessness, and agitation (95). Additionally, concentration and forgetfulness; sleep problems; irritability; worry about physical health; depression; depressive ideas and worry are symptom areas that have particular relevance for depressive disorders (96). Patten found there to be an increased risk of developing major depression with almost any long-term medical condition: 4% of those with one or more medical conditions, compared with 2.8% of those without medical conditions (97).

B. Diagnosis

Similar to IBS, there is no biological test for a diagnosis of MDD. A diagnosis of MDD is made by interpreting practice guidelines that are outlined in the American Psychiatric Association's Diagnostic and Statistical Manual, 4th edition (DSM-IV) (95). Although the diagnosis relies on the subjective judgment of the physician, most physicians agree on the use and application of the guidelines. However interpretation may lead to practice differences among physicians, as a patients' previous history as well as demonstrated behaviors during the current visit are used in determining a diagnosis of MDD.

A temporal criterion in the guidelines state that those affected by MDD must have experienced at least five or more symptoms during the same 2-week period. At least one of the symptoms must be either a depressed (or irritable) mood or loss of interest or pleasure. Other symptoms include: changes in weight or failure to make necessary weight gains; sleep problems; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or abnormal amounts of guilt; diminished ability to think or concentrate, or indecisiveness; and thoughts of death, including repeated suicidal ideation or plans for suicide, as well as suicidal attempts (98). These guidelines are a combination of clinical and historical consensus of experts in psychiatry about the most important signs and symptoms of depressive illness (95).

C. Recurrence

MDD is usually not a one time occurrence (99), with recurrence probabilities over 50%. In most individuals, MDD is episodic with multiple recurrences over the course of an individual's lifetime (100). A 15-year observational study of individuals with MDD at the beginning of follow-up and subsequently treated determined that after recovery the cumulative proportion of recurrence of any affective disorder at 15-years was 85% (Kaplan-Meier) (101). Specifically, the 15-year recurrence for MDD was 78% (101). Similarly, after 5 years of recovery, the cumulative probability of MDD recurrence was 60% (102). Thus MDD follows a waxing and waning of symptoms over the course of an individual's lifetime. Additionally, risk factors for recurrence of an affective disorder were being female, having an increased number of prior episodes, never marrying, and having a longer duration of episode before seeking treatment (101).

1.2.6 Epidemiology of Major Depressive Disorder

A. Public Health Significance/Burden of Illness

Depression affects 51 million individuals worldwide and is responsible for more than 1 in every 10 years of life lived with disability (103). The most common psychiatric disorder is MDD (95). In any one year, 10.3% of the US adult population is affected by depression (104). In the US the annual cost of depression has been estimated to be between $43.7 billion and $52.9 billion,

including costs due to health care, suicide, and workplace losses. Overall medical costs for a depressed individual are twice as high as a non-depressed individual. Depressed individuals utilize health care services 3 times more frequently and visit the emergency room 7 times more often. The diagnosis of depression may be missed in one third to one half of all patients (105).

Major depression is associated with increased symptom burden and decreased functioning and quality of life (106). Major depression is widespread and often chronic. Associated with depression are social and financial costs in the form of impaired relationships, lost productivity, and lost wages (105). Depression has a tremendous impact on all aspects of an individual's life (105). Depressed patients are more unlikely to comply with therapy than non-depressed patients, placing them at risk for poor health outcomes (105).

Major depression is a substantial public health burden as it is a common disorder associated with a high morbidity (107), high mortality (108, 109), and in the past it was largely untreated (107), with the percent of individuals receiving treatment increasing more recently. The meta-analysis of community-based studies by Cuijpers et al. determined that the combined relative risk (RR) of dying was 1.81 (95% CI 1.58-2.07) for depressed individuals compared to non-depressed individuals (109). Additionally, the

relative risk was significantly elevated for those individuals with subclinical depression (RR 1.65 (95% CI 1.39-1.96)) (109). The main drawback of this study was that information about chronic illnesses and other important confounders was not available for analysis (109). In Sweden, the standardized mortality ratios for patients with major depression were 2.0 (95% CI 2.0-2.1) for men and women for all causes combined (108). The main limitation of this study was that it only used patients with a hospital admission and thus the ratio may be higher than expected due to a possible selection of more severely depressed individuals (108).

B. Diagnostic Instruments

There are many different instruments that researchers use to diagnose and define depression, as well as to quantify its severity in clinical and epidemiological studies. These include the structured clinical interview for DSM-IV (SCID) (110), the composite international diagnostic interview (CIDI) (111), the structured psychopathological interview and rating of the social consequences of psychic disturbances for epidemiology (SPIKE) (112), the inventory for depressive symptomatology (IDS) (113), the research diagnostic criteria (RDC) (114), the schedule of affective disorders and schizophrenia (SADS) (115-117), the inventory to diagnose depression (IDD) (118, 119), the diagnostic interview schedule (DIS) (120), the short depression interview (SDI) (121), and the diagnostic inventory for depression (DID) (122), to name a few. Some of these are structured or semi-structured instruments, whereas

others are self-report instruments that were developed. All of these instruments are validated and have certain advantages and disadvantages associated with them. One of the problems in psychiatric research is comparing results of studies that use different instruments, as they may not necessarily obtain the same population of depressed patients based on the instrument used to operationalize a research definition of depression.

C. MDD Prevalence

Table 1.8 summarizes studies examining MDD prevalence. Prevalence rates vary by the time period for which prevalence is defined. Current prevalence rates vary between 1% and 6%, while six-month prevalence rates vary between ~2% and 5%. Lifetime and one-year prevalence rates are more variable, with one-year prevalence rates varying between ~2% and 12%, and lifetime prevalence rates ranging from 1.5% to 19%.

As these rates demonstrate, there is much variability in their ranges. Reasons for this are the way in which MDD is defined, and also cultural or environmental differences. First, while most of these studies used either the DIS or the CIDI to define MDD by using the responses to obtain Diagnostic and Statistical Manual (DSM) MDD diagnoses, differing versions of the DSM were used in different studies. Thus, variability in the estimates is introduced through these definitional differences.

Another possible cause of the demonstrated variability may be cultural or environmental differences. This is demonstrated in the studies used in the reviews by Bland et al. (123) and Weissman et al. (124). The included studies of these reviews were performed in the US, Puerto Rico, France, Italy, Lebanon, Korea, Taiwan, and New Zealand; and the lifetime prevalence rates were all standardized to the US population. Thus, by standardizing these rates, more similar lifetime prevalences should have been demonstrated if no cultural/environmental influences have an effect on MDD. However this was not the case and therefore cultural or environmental influences have an effect on MDD prevalence and MDD susceptibility. Despite these limitations, the majority of studies in Table 1.8 estimate the range of the lifetime prevalence of MDD to be between 10-20%, with some additional outliers.

D. MDD Risk Factors

The major risk factors for major depression are being female (95, 107, 120, 125-127), having stressful life events (95, 127), experiencing adverse childhood experiences (95, 127), demonstrating certain personality traits (95, 127), being born after World War II (107, 120, 125, 126), being separated/divorced or in an unhappy marriage (107, 126), having asthma or heart disease (126), and having a family history of major depression (107, 127). Based on these risk factors, it follows that cohort effects may also be influencing MDD (107, 120). Such an example is individuals born after World War II. Additionally, individuals born in the same birth cohort endure the

same social and political life stresses. With major life stresses, such as war, affecting a birth cohort, additional effects may also been seen in MDD occurrence.

Age. Depression can affect both adults and children. Evidence of an increased rate in younger individuals has been demonstrated, with the average age of first onset in young adulthood (107, 123). The National Comorbidity Study—Replication (NCS-R) was consistent with results from previous studies that demonstrated that major depressive disorder has an early onset (111).

Gender. Of the 10 to 14 million individuals who are depressed in any given year (105), women aged 18 to 45 years comprise the largest group (128). Women have consistently demonstrated a higher risk of MDD than men, and in most studies the ratio of prevalence rates in women to men has been between 1.5 and 2.5:1 (95, 127). For example, the National Comorbidity Study estimated a lifetime prevalence of MDD in US women to be 21.3%, and 12.7% in US men (129). Additionally, data from the National Survey of Psychiatric Morbidity, completed in Britain, found that women suffer from higher rates of depression than men until menopause and then the rate of depression in women becomes similar to men (96).

Stressful life events. In the community study by Patten et al., experiencing one or more recent life events was associated with the 12-

month prevalence of major depression (OR 2.01, 95% CI 1.08-3.72) (125). Additionally Kessler et al. demonstrated that the exposure to stressful life events is higher among individuals with a history of depression (127). In the developmental model of major depression described by Kendler et al., stressful life events in the last year had a correlation of 0.35 with an episode of major depression in the last year (130).

***Marital factors*.** In an Australian community sample, the 30-day prevalence of major depression was less in those currently married (OR 1.0) than in those never married (OR 1.77, 95% CI 1.19-2.63) or those separated, widowed, or divorced (OR 2.81, 95% CI 1.53-4.82) (126). Weissman et al. observed the lowest major depression rates among married women and women that get along with their spouse, and the highest rates of major depression among women in unhappy marriages (107). In fact, unhappy marriage increased the risk of major depression about 25-fold in both men and women (107). Additionally, in the developmental model by Kendler et al., marital problems or difficulties in the last year and history of divorce were correlated with an episode of major depression in the last year (130).

***Family history*.** A two-to-threefold increased risk for major depression has been demonstrated if there is a family history of the disorder (95, 105, 107, 125). The Zurich cohort reported that of participants receiving a diagnosis of depression, 54-73% reported a family history of depression

among first degree relatives (112). Recently, results from family studies are consistently showing that major depression is familial (107). Additionally, children of depressed parents are more than 3 times as likely to experience depression than are children of non-depressed parents (105).

1.2.7 Pathophysiology of Major Depressive Disorder

Depression is a symptom of many medical conditions, including stroke, Cushing's disease, hypothyroidism, multiple sclerosis, Huntington's disease, and Parkinson's disease (95). In addition, it is commonly comorbid with numerous psychiatric disorders such as attention-deficit/hyperactivity disorder, bulimia nervosa, dysthymic disorder, generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder, premenstrual syndrome, and social phobia (131); however its etiology is not fully understood.

A. Biologic Abnormalities in MDD/Possible Etiologies

Some of the biological bases of MDD are dysregulations of circadian rhythms, cognitive processing, and both appetite and psychomotor functioning, which may be an influence of the co-occurring medical conditions (95). Major depression patients usually experience abnormalities in mood, sleep, sexual behavior, and appetite (132-134). These disturbed functions are regulated by serotonin and norepinephrine (132-135). Additionally, monoamine oxidase (MAO) is one of the major enzymes responsible for the

degradation of neurotransmitters such as serotonin, norepinephrine, and dopamine (136). Significant symptom improvement in mood and anxiety disorders can occur by the administration of substances that inhibit MAO activity (136) or by pharmaceuticals that increase serotonin activity by decreasing its reuptake, known as selective serotonin reuptake inhibitors (SSRIs) (137).

The exact causes of depression are still being investigated (138); but likely both genetic and environmental factors contribute to its pathogenesis (139). A plethora of information about the biology of depression comes from results of antidepressant drug studies (140, 141). Biologic causes include changes in the chemistry of the brain or fluctuations in the body's secretion of hormones (139). Individuals with depression may have abnormally low levels of certain brain chemicals and slowed cellular activity in areas of the brain that control mood, appetite, sleep, and other functions (138). Additionally, increases or decreases in the production of specific hormones may interfere with the brain's natural chemistry and lead to depression (138, 140).

B. Role of Neurotransmitters

Biologically, the brain communicates with the body through the work of neurotransmitters (138-140). Neurotransmitters carry the brains messages across synapses from one neuron to another (138, 139). Each neurotransmitter has a distinctive structure, which allows for recognition of its

receptors structure (similar to a lock and key). If the receptor is not the specific receptor corresponding to that neurotransmitter, its message is not relayed across the synapse (139). Once the neurotransmitter has bonded to its receptor and delivered the message, the neurotransmitter pulls away from the receptor and returns to the synapse, where the brain is able to remove the neurotransmitter (138, 139).

C. Neurotransmitters Implicated in MDD

Of the many known neurotransmitters in the brain, associations between depression and the activity of three main neurotransmitters: norepinephrine, serotonin, and dopamine are demonstrated in the literature (139). These neurotransmitters are active in the brain areas that control activities shown to malfunction during depression (135, 139). In MDD a dysfunction of neural function in both pre- and post-synaptic serotonergic systems is demonstrated (137). In addition to the neurotransmitters having a role in depression, it is also thought that their receptors may be involved in causing depression (139).

Norepinephrine (NE). Norepinephrine plays a role in modulating mood, sleep, appetite, and neuroendocrine functions which are often abnormal in MDD (142, 143). The norepinephrine transporter (NET) is a main target of antidepressant action and is dysregulated in major depression (134, 144). NET is responsible for terminating the action of NE in the synapse

(144). Blockade of NET by tricyclic antidepressants results in prolongation of the action of NE in the synapse (140), as antidepressant treatment causes decreased NE turnover (145). Lee et al. demonstrated that levels of NETs on brain noradrenergic neurons appear to be regulated in such a way as to maintain normal concentrations of NE in the noradrenergic synapse (146). Thus malfunction of the NET may be involved in the etiology of MDD (142).

Data from a study using postmortem brain tissue demonstrated a reduced expression of the NET on noradrenergic neurons of the locus coeruleus in individuals with major depression, using a radioisotope binding technique (144). Additionally, increased levels of NE and its metabolites were reported in cerebrospinal fluid, plasma, and urine of depressed patients over normal controls (147-149). Therefore, these studies demonstrate that NE and the NET are dysregulated in individuals with major depression.

Serotonin (5-HT). Substantial evidence supports a role for dysfunction of brain serotonergic (5-HT) systems in the pathogenesis of major depression (115), including the serotonin type 5 receptors, serotonin type 2 receptors, serotonin type 3 receptors, serotonin type 1 receptors, and the serotonin transporter. The relative abundance of serotonin type 6 receptor (5-HT_6) in the limbic area of the brain and the high affinity of some antidepressants for the 5-HT_6 receptors suggest that this receptor might be involved in the pathogenesis of mood disorders (150). Serotonin type 2

receptor (5-HT_2) density and function are disturbed in the depressed patient and return to normal values only following effective treatment (140). A decrease in the functional activity of the 5-HT_{2A} receptor is associated with the symptoms of depression (140). It was initially thought that the main function of 5-HT was as a peripheral hormone due to the relatively high concentration in the gastrointestinal (GI) tract and in blood (140), however there is increasing evidence suggesting that the peripheral serotonin receptor is identical to the serotonin type 3 (5-HT_3) receptor in the brain (140), thus there is a link between the serotonin in the brain and the serotonin in the GI tract which may explain the brain-gut dysfunction demonstrated in IBS.

Although the precise mechanism of action of most antidepressants is incompletely understood, there is evidence to suggest that serotonin receptors, particularly 5-HT_{1A} and 5-HT_2, play a role in the action of antidepressants (140). Stimulation of 5-HT_{1A} pre-synaptic receptors results in a decrease in the excitation of serotonergic neurons (135, 151, 152), thus a possible cause of depression. Depressed patients have decreased 5-HT_2 receptors in the brain in comparison to control participants (153). Irrespective of the presumed specificity of antidepressants on the various neurotransmitters in the brain, in general antidepressants modulate the activity of a number of different transmitters whose functions are inter-related (140). There is some evidence to suggest that serotonergic function is abnormal in some groups of depressives, yet it is not certain if such changes

are a result or a cause of the mood states associated with major depressive disorder (137, 140).

D. Serotonin Hypothesis of MDD

The 'serotonin hypothesis' of MDD is the most widely accepted hypothesis of the neural basis of MDD (137). Serotonin is synthesized in the body from the essential amino acid L-tryptophan, provided to the body by protein-rich food (152). Figure 1.1 diagrams the metabolic pathways of tryptophan (152). In individuals with MDD, a decrease in the concentration of serotonin and an increase in the concentration of kynurenine has been noted. This implies that there is a relative deficiency of brain serotonergic activity in depression (151, 154), attributable to either less serotonin release or to fewer serotonin receptors or impaired serotonin receptor-mediated signal transduction (135). As Staley et al. have summarized, support for the serotonin hypothesis comes from studies that have measured 5-HT function in various ways: from cerebrospinal fluid (CSF) 5-Hydroxyindoleacetic acid (5-HIAA) levels (155, 156), plasma precursors (140, 156), and blood platelet function (141, 156).

Additional evidence for this hypothesis comes from studies of the serotonin transporter and other serotonin receptor subtypes. The 5-HT transporter (SERT) is located on the pre-synaptic membrane and controls the amount of 5-HT in the synapse (141). The SERT is responsible for the

reuptake of 5-HT from the extracellular space back into the neuron (157), which thereby inactivates serotonin (151). The 5-HT that the SERT transports back into the presynaptic neuron is then stored in vesicles until it is either re-released into the synapse or it is degraded (151). Arango et al. document that MDD sufferers have fewer platelet SERT sites (157). Moreover, individuals with MDD have less SERT binding compared to nondepressed individuals, with the difference in SERT binding more pronounced in men (33%) than in women (19%) (157), which may explain part of the gender difference observed in MDD.

Adequate control of the serotonin network depends on appropriate balance of presynaptic 5-HT storage and release as well as on 5-HT reuptake from the synaptic cleft by 5-HT transporters (152). The release of serotonin from the neuron is necessary to activate its postsynaptic receptors which activate the corresponding second messenger systems or stimulate target organs (152). A schematic of a serotonin neuron is portrayed in Figure 1.2. Recently, Linner et al. demonstrated that noradrenaline may regulate serotonergic neurotransmission at both the serotonin neuron and the nerve-terminal (158). At the serotonin neuron, regulation by noradrenaline is accomplished by stimulatory α_1-adrenoceptors, whereas at the nerve-terminal, regulation of serotonin transmission is inhibited by noradrenergic α_2-adrenoceptors (158). Thus while the biologic mechanism of MDD is evolving,

the current state of knowledge is implicating serotonin as a major contributor to the biologic basis of MDD.

1.2.8 Role of Genetics in Major Depressive Disorder Etiology

The complex etiology of MDD is studied with family, adoption, and twin studies. Additionally, MDD has been the subject of linkage studies and association studies, mainly searching for gene regions linked to MDD and candidate genes that are associated with neurotransmitters that are MDD drug targets or hypothesized to be involved in the etiology of MDD. These studies are summarized below.

A. Family Studies

Table 1.9 summarizes family studies examining the familiality of MDD with adequate methods, as outlined in the meta-analysis by Sullivan et al. (159). These studies used a mix of population- and clinic-based samples, thus allowing the comparison of possible treatment-seeking effects on the estimates. The phenotype of MDD is well-defined in these studies, as MDD was distinctly separated from bipolar disorder. All probands were recruited systematically, and their diagnoses were made blind to recruitment status. Additionally, the relatives were diagnosed without knowledge of the affection status of other relatives, and through a direct interview, where possible.

Sullivan et al. combined the first five studies in Table 1.9 into a meta-analysis, and the appropriate homogeneous combined odds ratio for proband

versus first-degree relative status was 2.84 (95% CI 2.31-3.49) (159), supporting the notion of the familiality of MDD. Since publication of that meta-analysis, additional population-based family studies of MDD probands were performed. This is in contrast to prior studies which used mainly hospital or clinic samples for probands, which was a limitation of the meta-analysis by Sullivan et al. (159).

The population-based studies also demonstrate familiality of MDD in the following manners: children of depressed probands having a higher risk of MDD (160-162); the rate of MDD in relatives of adolescent probands being elevated (163); and relatives of chronic MDD probands exhibiting higher rates of MDD than either relatives of episodic MDD probands or relatives of probands with no history of mood disorders (164). Thus both the results of the meta-analysis and the results of subsequent population-based family studies support the hypothesis of the familiality of MDD.

B. Adoption Studies

Table 1.10 summarizes the three MDD adoption studies. These studies are not as methodologically rigorous as the aforementioned family studies; however a discussion is warranted. The study by von Knorring et al. (165) did not support a genetic influence to the liability of MDD, however the definition of the MDD probands was not rigorous, employing health insurance, sick leave, and claims data. This is in methodologic contrast to the additional

adoption studies, one which used direct interview (166), and the other employed discharge diagnoses and death records (167) to define MDD probands, both more rigorous methodologies. The study by Cadoret et al. (166) had limited statistical power as well as limited information on the biological parents of the adoptees, and when the data was analyzed by gender, the results were non-significant (166). However Sullivan et al. analyzed the relationship combining genders and found a significant trend-level relationship (OR 2.54; 95% CI 1.0-6.47) (159). The adoption study by Wender et al. (167) provided the best evidence for a genetic effect regardless of the fact that the MDD information came from an indirect source as opposed to direct interviews (OR 7.25, 95% CI: 1.21-43.20) (159), however the width of the confidence interval demonstrates the lack of precision due to a small sample size. Thus it appears as though the majority of these adoption studies support the genetic influence to MDD (159).

C. Twin Studies

Table 1.11 summarizes the twin studies performed for MDD. These studies fulfilled the criteria stated of family studies, with the additional criteria that zygosity was determined accurately for all twins, both those with and without MDD, as fully stated in the meta-analysis by Sullivan et al. (159). These studies used both community-based and clinic-based samples of twins, and employed traditional twin methodologies, MZ and DZ concordance rates, as well as twin modeling. All of these studies used DSM-III-R criteria

for MDD based on interviews, and the majority were community-based samples. Sullivan et al. (159) combined the first six studies in Table 1.11 into a meta-analysis. By inspecting the difference in concordance rates between MZ and DZ twins within these studies, MZ twin concordance rates are usually 1.5-2 times greater than DZ twin concordance rates, implying a genetic influence to MDD (86).

Methodologically twin studies are able to partition the variance in liability to MDD into three components: additive genetic effects (quantitatively known as heritability), common environmental effects (shared family/twin environment), and unique environmental effects (individual environment) (159). The meta-analysis by Sullivan et al. obtained a combined estimate of heritability of 0.37 (95% CI 0.33-0.42) (159) and the combined estimate for the unique environmental effects was 0.63 (95% CI 0.58-0.67) (159). The model used to obtain these estimates determined that the common environment did not significantly contribute to the variance in liability to MDD. Since the publication of this meta-analysis, two additional twin studies were performed satisfying the inclusion criteria of the meta-analysis. In these two studies, the estimated heritability was between 0.34 and 0.40 (168, 169) and the remainder of the variance in liability to MDD was due to unique environmental effects of the individual, similar to the results of the meta-analysis. Additionally, the meta-analysis determined the heritability in clinical studies to be 0.43 (95% CI 0.21-0.58) (159) and 0.37 (95% CI 0.28-0.42) in

community-based samples (159), which are not markedly different from each other.

D. Linkage Studies

Table 1.12 summarizes linkage studies performed for MDD. These studies were defined by families with MDD or recurrent, early-onset MDD (RE-MDD) and most were genome-scans. Additionally, the majority used standardized instruments to obtain valid MDD diagnoses. An issue with many of these studies is the use of RE-MDD to identify the probands, which may be a genetically distinct subtype of MDD.

The majority of studies did not find significant evidence for linkage to MDD; however some found evidence for linkage that was gender dependent. Among women chromosome 2 demonstrated significant linkage for RE-MDD (170, 171), whereas men demonstrated linkage to chromosome 12 for MDD (172). Two recent studies report significant linkage of RE-MDD to chromosome 11 (multi-point LOD 4.2, $p<0.001$ for marker D11S1984; and LOD 2.51, $p<0.001$ for marker D11S2002) (173) and to chromosome 15 (LOD 3.73, $p=0.023$, flanked by markers D15S652 and D15S816) (174), independent of gender. These results do not overlap with the regions implicated previously for specific gender. Reasons for this discrepancy may be that a 'clearer' signal may have been available when examining each gender independently. Additionally, different markers and families were used

in these studies, and thus replication in different families is needed. Lastly, the lack of overlapping regions may be due to none of these regions being the disease locus, but rather being susceptibility loci (173). Further it is possible that since MDD is a complex disease that these regions may all be important in the etiology of MDD. Regardless, these studies demonstrate regions of the genome having an effect on MDD, with RE-MDD deemed to be the most genetic subtype of MDD.

E. Candidate Gene Studies

Table 1.13 displays information on candidate gene association studies of MDD. Most of these studies did not examine the same gene, however many studies have examined *SLC6A4* and various serotonin receptors. These candidate genes are involved in the biochemical neurotransmission pathways hypothesized to cause depression, or are the site of antidepressant drug activity (175). These studies used both in- and out-patients diagnosed with MDD through use of interviews and case notes to obtain DSM-IV MDD diagnoses.

The following genes demonstrated positive associations with MDD: *SLC6A4(I/D)* (176-178), *SLC6A4(VNTR, allele9)* (177, 179), *SLC6A4(VNTR, allele12)* (180), *SLC6A4(VNTR, allele2)* (181), *PLA2* (182), *TPH1* (183), *DRD4* (184), *HTR1B* (185), *CYP2C9*3* (186), *HTR2C* (115), *MAOA* (116, 187), *CRHR2* (188), *TPH2* (189), *GABRA3* (190), *TH* (191, 192), *HTR2A*

(193), and *GNAL* (194). The majority of progress is in the serotonin system, although most findings have no replication and suffer from inadequate power. A meta-analysis for the *SLC6A4 (I/D)* polymorphism demonstrated a non-significant summary effect of the polymorphism on the risk of MDD (OR 1.08, 95% CI 0.96-1.22, p=0.198) (195). The meta-analysis further pooled results of prior studies for the *SLC6A4 (VNTR)* polymorphisms. Overall, none of the alleles (9, 10, or 12) showed an increased risk for MDD (195), results in Table 1.14. The results of the meta-analysis should be considered preliminary, as it combined results from various regions of the world and used differing diagnostic instruments for the diagnosis of MDD. Thus future studies should be larger, aim to replicate initial positive findings in the same geographic region, use similar diagnostic instruments and criteria for MDD, and investigate other genes with hypothesized involvement in the pathophysiology of MDD.

***Limitations*.** A problem with many of these studies is control selection. Many controls were not screened for psychiatric disorders. Additionally, all these studies are underpowered to detect small effects (OR of 1.2-1.3). The type II error rate is also large due to multiple comparisons. Spurious associations are also a concern when the cases and controls are not matched properly (86). Misclassification of the genotype (exposure) is possible due to laboratory errors (196), although these are becoming less. Disease misclassification may have an effect due to disease heterogeneity,

phenocopies, and age-related penetrance (93). Specifically for MDD, relying on DSM criteria for disease classification causes problems because the diagnosis is based on fulfillment of these criteria and not on biology or biological tests (154), and this may dilute a true effect by causing heterogeneity by including as diseased individuals that may have a different causal pathway for their disease. Lastly, ethnicity may have an affect on the results for some of the polymorphisms, as allele frequencies vary between different populations/ethnicities (94). However the majority of these studies were completed in one ethnicity and thus within study population stratification is minimized, but between studies there is evidence of population stratification as differing results were demonstrated in different populations. This is clearly demonstrated for *SLC6A4 (VNTR)* where significant results were found among English/Scottish (179), and Han Chinese (180), but non-significant results were demonstrated among Germans (197), Japanese (198, 199), French (200), Spanish (201), and Danish (202).

1.2.9 Lifetime Co-morbid IBS and MDD

A. Definition of 'Lifetime Co-morbid'

For this study, the phrase 'lifetime co-morbid' means that an individual has had both disorders at some point in their lifetime, not necessarily at the same time. This is due to the chronicity as well as the waxing and waning of

both IBS and MDD symptoms, and the fact that the causality between IBS and MDD is not known.

B. Epidemiology of Co-morbid IBS and MDD

Psychological disorders are common co-morbid conditions in most IBS patients who actively seek subspecialist medical care (7). Patients with major depression and any IBS were more likely to have a personal or family history of bowel disease, but not more likely to be treated for their gastrointestinal problems (39). Among elderly, slow colonic transit in constipated individuals has been associated with increased psychological symptoms (203).

There have been a number of studies which captured information regarding psychiatric disorders in IBS patients and vice versa. In these studies, there is some consensus among the findings, but also a few inconsistent findings, as summarized below. The largest consensus is that there is an overlap of MDD and IBS, where approximately 50% of the sufferers of either disorder have the other disorder as well.

In two studies of IBS patients, one using a structured interview (37) and one using a self-rating scale for depression (38), it was found that IBS patients have psychopathology that is diagnosed more often and are more anxious and depressed than either inflammatory bowel disease (IBD) patients or healthy controls (37). In addition, depression scores were significantly higher among patients with IBS compared to controls (38).

Mayer et al. have summarized studies of IBS patients seeking care at GI clinics and found that in studies with an adequate sample size and a standardized psychiatric interview, 50-60% of IBS patients also have psychiatric disorders (204). The most commonly observed psychiatric disorders found in these IBS patients are major depression, panic disorder, social phobia, generalized anxiety disorder, post-traumatic stress disorder, and somatization disorder (205). There is some evidence that depression is more common in patients suffering from chronic IBS symptoms, and anxiety may be more prominent early in the course of IBS (204, 205). Of IBS patients seeking specialist care, 54-94% met Diagnostic and Statistical Manual, third edition revised (DSM-III-R) criteria for a primary (Axis I) psychological disorder (206), but only 18% of IBS patients in a population-based study met Diagnostic and Statistical Manual, third edition (DSM-III) criteria for a primary psychiatric disorder (207). Of psychiatric disorders, major depression has been shown to precede IBS or partial IBS in 50% of subjects, and to follow IBS in 45.8% of subjects with the remaining 4.2% having IBS and psychiatric disorders diagnosed at approximately the same time (39).

Additionally, in a study of psychiatric patients, the point prevalence of IBS was 29% in patients with major depression and 37% in generalized anxiety disorder patients as compared to 11% in the controls (39). In a recent population study in New Zealand, the association between MDD and IBS was not substantiated (29). This was the first study to define IBS using Rome II

criteria, which may have had an effect on the association since these are the most restrictive IBS criteria. However, the authors concluded that the high rates of psychiatric disorders in IBS patients reported in prior studies using referral centers probably reflects referral bias (29).

In population-based samples, subjects who reported two GI symptoms had significantly higher lifetime prevalence rates for depression, panic disorder, and agoraphobia than those who reported no GI symptoms (204). In a community study, lifetime psychiatric disorders are significantly more common in individuals meeting diagnostic criteria for IBS (63%) than in those without IBS (24.8%) (205). Additionally, the association of IBS and psychiatric disorders appeared to be independent of treatment seeking status (205). Similarly, in the IBS review by Camilleri et al., more psychiatric disease was observed in the general population of IBS sufferers implying that the increase in psychiatric disease among IBS sufferers was not due to health care seeking bias (208).

C. Limitations of Prior Research

These studies are problematic and more research on the co-occurrence of MDD and IBS is needed. The majority of studies on co-occurrence have not been population-based, rather only considering patients which have sought treatment. Thus they may not be representative of all individuals with IBS and MDD and may suffer from referral bias as well as

treatment-seeking bias. Also, the diagnostic criteria used to define IBS were not specified in many of the studies, although it could be assumed to be either Rome I or Manning based on the date of the studies. In one study, neither of these criteria were used, but rather criteria that were referred to as 'Drossman criteria' (39). Therefore, using the more recent Rome II criteria and a population based sample could make the results more generalizable to the population of individuals with IBS, both those that do and do not seek treatment.

1.2.10 Role of Genetics in Lifetime Co-morbid IBS and MDD

A. Familial Overlap of IBS and MDD

There have been 5 studies examining the familiality of IBS, as discussed in detail on page 19. Similarly, there are at least 11 family studies of MDD, discussed previously on page 45. From these studies, there is support of a familial (environmental or genetic) component to both of these disorders independently. Additionally, it has been shown that patients with major depression and any IBS were more likely to have a personal or family history of bowel disease but not more likely to be treated for their gastrointestinal problems (39).

The familial overlap of IBS and major depression was demonstrated in a study conducted by Sullivan et al. (209). This study showed that patients with both IBS and major depression had a similar, higher prevalence of

relatives with psychiatric illness than controls, attributable to a higher prevalence of anxiety and depressive disorder in the relatives (209). Additionally, Hudson et al. remarked that relatives of individuals with IBS display a higher prevalence of major affective disorder than relatives of control subjects (210).

In a small family study by Woodman et al., IBS probands (defined with Manning criteria) had a greater frequency of lifetime psychiatric disorders than control probands, and this was also demonstrated when major depression was separated out from other psychiatric disorders (211). This study also demonstrated familiality of IBS and MDD because relatives of IBS probands demonstrated more lifetime depression than relatives of control probands. A drawback of this study, aside from its small size, is that it is not population based and thus it is not known if the psychiatric disorders in IBS probands contributed to their treatment seeking (211).

B. Treatment Response Subgroups

The prevailing thought in research on co-occurrence is that response to pharmacologic treatments may identify groups of disorders with a common pathophysiology (210). Support for this hypothesis is from patients demonstrating an improvement in their co-occurring disorders with several different classes of antidepressant drugs which are chemically unrelated. The disorders may share a specific biologic abnormality, either known or

unknown, in common with major depression, and thus the various classes of antidepressant drugs may benefit the disorders via their effect on the particular biologic abnormality in common (212).

Specifically with regard to IBS and major depression, patients with IBS, regardless of the presence of psychiatric symptoms, respond to three chemically different classes of antidepressant medications. These are tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (8, 14, 210).

C. Serotonin to Treat IBS and MDD

Indirectly, the therapeutic use of serotonin to treat both MDD and IBS independently suggests the possibility of a common etiology, and further a possible common genetic or environmental etiology. Recently, investigation into the role of serotonin in the gut of IBS sufferers has begun (8), as discussed previously on page 17. Additionally, serotonin is also involved in treating major depression, as one of the major classes of drugs used for treatment is selective serotonin reuptake inhibitors (SSRIs). Thus, serotonin abnormalities may represent a common pathophysiology between IBS and various disorders often found to be co-morbid with IBS, such as depression, anxiety disorders, fibromyalgia, migraine, drug abuse, and eating disorders (53, 54, 131).

The function of the serotonin transporter protein (*SLC6A4*) is to remove 5-HT from the synaptic cleft and determine the magnitude and duration of postsynaptic receptor-mediated signaling, thus playing a pivotal role in the fine-tuning of 5-HT neurotransmission (213). Serotonin transporter functions are dysregulated in depression (142).

A polymorphism in the promoter for the synthesis of the serotonin transporter gene, *SLC6A4,* is known to influence the response to serotonergic medications in depression. Recently, genetic polymorphisms (long, short, and heterozygous) at the *SLC6A4* promoter have been shown to influence response to a 5-HT_3 antagonist in diarrhea-predominant IBS (76). *SLC6A4* polymorphisms were associated with colonic transit response, specifically, there was a greater response to 5-HT_3 antagonists in those with long homozygous versus heterozygous polymorphisms (76). Thus *SLC6A4* has demonstrated biological importance in both IBS and MDD.

D. Summary

Through this review of the literature, commonalities between IBS and MDD exist and have been highlighted. For IBS, family, twin, and population based studies demonstrate that both genes and environment are important in its etiology. Likewise in MDD the relevance of genes and environment to its etiology is demonstrated in family, adoption, twin, and population based studies. Additionally, IBS and MDD have a demonstrated tendency to affect

the same individual over the course of their lifetime. Further, a commonality in their possible etiologies, namely the influence of the neurotransmitter serotonin, suggests that the demonstrated co-occurrence may not be spurious but possibly due to a common deficiency or dysfunction. Thus common genetic and/or environmental effects may explain the demonstrated covariation between IBS and MDD.

1.3 TABLES

Table 1.1: Manning, Rome I, and Rome II Diagnostic Criteria for Irritable Bowel Syndrome.

Manning Criteria (27)	**Rome I Criteria (8)**	**Rome II Criteria (8)**
Two or more of the following symptoms	At least 3 months of continuous or recurrent symptoms of abdominal pain that is:	12 or more weeks of continuous or recurrent abdominal pain or discomfort
Pain eased after bowel movement	Relieved by defecation **And/or**	**Plus** at least 2 of the following:
Looser stools at onset of pain	Associated with a change in stool consistency **And/or**	Relieved by defecation **And/or**
More frequent bowel movements at onset of pain	Associated with a change in frequency of stool	Associated with change in frequency of stool **And/or**
Abdominal distension	**Plus** 2 or more of the following greater than 25% of the time:	Associated with a change in form (appearance) of stool
Mucus per rectum	Altered stool frequency (more than 3 per day or less than 3 per week)	
Feeling of incomplete emptying	Altered stool form (lumpy, hard or watery, loose)	
	Altered stool passage (straining, urgency, or incomplete evacuation)	
	Passage of mucus	
	Bloating or feeling of abdominal distension	

Table 1.2: Red flags in diagnosing Irritable Bowel Syndrome.

Anemia
Family history of colon cancer or inflammatory bowel disease
Fever
Heme-positive stools
New or recent onset in patient older than 50 years of age
Nocturnal symptoms
Palpable abdominal or rectal mass
Persistent diarrhea or severe constipation
Recent antibiotic use
Rectal bleeding
Weight loss

Table 1.3: IBS prevalence stratified by diagnostic criteria.

Criteria	Prevalence Range	Reference
Manning	10.3-20.4%	(5, 9, 23, 24, 28, 29)
Rome I	8.5-12%	(23-25, 28)
Rome II	3.3-7%	(5, 23, 26, 29-31)

Table 1.4: International population-based IBS prevalence ranges.

Country	Sample Size(s)	Manning Range	Rome I Range	Rome II Range
Australia/New Zealand (5, 28, 29)	730; 2910; 890	12.7%-13.6%	4.4%-12%	4.3%-6.9%
Bangladesh (25)	2426		8.5%	
Canada (21)	1149		13.5%	12.1%*
Europe (UK, France, Germany, Italy, Holland, Belgium, Spain, Switzerland) (10)		6.5%	4.2%	2.9%
Hong Kong (20, 30)	1649; 1000		4.1%	6.6%
Spain (23)	2000	10.3%	12.1%	3.3%
United States (9, 24)	3022; 643	18-20.4%	8.5%	

*modified Rome II criteria

Table 1.5: Family Studies of IBS*.

Authors	Setting	Sample size	IBS definition	Method of family member assessment	Adjustment variables	Results
Oster, J (214)	School children	18,162 over 8 years of follow-up 635 children examined each year for 5 or more consecutive years 185 had abdominal pain for 3 or more consecutive years	Abdominal pain	Questionnaire sent to parents of case and control children		Children with pain came from families where parents were prone to pain OR 2.29 (95% CI 1.69-2.89)
Whorwell, PJ, et al. (84)	IBS outpatients	100 IBS patients 100 matched controls	Abdominal pain, abdominal distension, and abnormal bowel habit in association with normal hematology, serum biochemistry, rectal history, and colonoscopy or contrast radiology	No direct assessment Participants asked if there was a family history of IBS	Age, gender, social class	Familial incidence of IBS is 33%

Table 1.5 (Continued): Family Studies of IBS*.

Authors	Setting	Sample size	IBS definition	Method of family member assessment	Adjustment variables	Results
Locke, GR, et al. (60)	Population-based cohort in Olmsted County, MN	643 (76 with IBS) First degree relatives With abdominal Symptoms, 148 Without abdominal Symptoms, 475 Spouse Present abdominal Symptoms, 132 Absent abdominal Symptoms, 475	Rome I	Self-report questionnaire asked about GI symptoms of spouse and 1st degree relatives history of abdominal pain or bowel problems	Age, gender, psychosomatic symptoms	IBS prevalence 12% When abdominal symptoms were present in first degree relative the odds of IBS were twice as great (OR 2.3; 95%CI 1.3-3.9), adjusted for age, sex, and psychosomatic symptom checklist score

Table 1.5 (Continued): Family Studies of IBS*.

Authors	Setting	Sample size	IBS definition	Method of family member assessment	Adjustment variables	Results
Kalantar, JS, et al. (82)	IBS patients and IBS people in educational classes (Olmsted County, MN)	181 IBS patients 153 relatives of patients 98 spouse relatives 97 children	Manning or Rome I (Manning used for analysis since all Rome I also fulfilled Manning)	Participants asked for name and address of relatives and then relatives sent Bowel Disease Questionnaire	Age, gender OR attenuated when additional control from somatization score	IBS prevalence of 17% in patients' relatives IBS prevalence of 7% in spouses' relatives OR 2.72 (1.19-6.25)
Kalantar, JS, et al. (83)	Longitudinal population-based study in Olmsted County, MN	523 total 404 controls 119 IBS	Rome I	Participant questionnaire asked specific questions about 1st degree relatives abdominal aches, pains, or bowel problems	Age, gender, somatic symptom score	IBS prevalence 11-12% Positive family history of IBS in 23% of subjects Increased odds of persistent IBS among those with a family member with symptoms, OR 2.51 (95% CI 1.28-4.90) Increased odds of fluctuating IBS among those with a family member with symptoms, OR 2.38 (95% CI 1.30-4.37)

*IBS= irritable bowel syndrome; OR= odds ratio; 95% CI= 95% confidence interval; GI=gastrointestinal

Table 1.6: Twin studies of IBS*.

Authors	Setting	Sample size	IBS definition	Adjustment variables	Results
Morris-Yates, A, et al. (91)	Volunteer twins in Australian twin registry	462 twin pairs (924 twins)	Functional bowel disorder (greater than 1 symptom)	Gender	MZ IBS concordance 33.3% DZ IBS concordance 13.3% Genetic liability to FBD estimated at 58% A^2=0.754 C^2=0.657
Levy, RL, et al. (61)	Population-based registry of twins in the Commonwealth of Virginia	6060 twin pairs (10,699 twins) 281 pairs had 1 or both twins with IBS	Self-report of IBS diagnosis	Father with IBS, Mother with IBS, co-twin with IBS	IBS prevalence 2.6% MZ IBS concordance 17.2% DZ IBS concordance 8.4% Having either a mother or father with IBS is greater risk of IBS than having a twin with IBS
Svedberg, P, et al. (90)	Population-based registry of twins in Sweden	850 twin pairs (58 twin pairs discordant for IBS, 72 unrelated IBS cases)	Diagnostic algorithm that combined abdominal problems at least 7 days a month with 1 additional IBS symptom	Case-control study: Age, gender	Case-control study demonstrated associations between 5 disorders and IBS Co-twin analysis determined that three of these disorders may be due to common genetic effects between IBS and the disorder

Table 1.6 (Continued): Twin studies of IBS*.

Authors	Setting	Sample size	IBS definition	Adjustment variables	Results
Mohammed, I, et al. (92)	Population-based registry of twins in the United Kingdom	1870 twin pairs (888 MZ twin pairs and 982 same sex DZ twin pairs)	Self-report mailed questionnaire; past-year Rome II IBS	Matched for age, drug therapy, excess alcohol, psychosomatic score, and handedness	MZ IBS concordance: 28% DZ IBS concordance: 27% Twin modeling revealed genetic factors of little or no influence on IBS

*MZ= monozygotic twin; DZ= dizygotic twin; IBS=irritable bowel syndrome; FBD= functional bowel disorder; a^2=additive genetic effect; c^2=common environmental effect

Table 1.7: Genetic Association Studies for IBS*.

Gene (Polymorphism)	Population	Study	Size	Results	Reference
TGFB1 promoter (*C-509T*)	IBD/ Crohn's Disease	Association	141 IBD 88 Healthy controls	NS	Schulte, CMS, et al. (215)
SLC6A4 (*VNTR* and *5-HTTLPR*)	IBS (Rome I)	Association	54 IBS 91 Healthy controls	NS	Pata, C, et al. (81)
SLC6A4 (*SERT-P*)	Diarrhea-predominant IBS (Rome I)	Treatment response	30 IBS patients (23 with genotyping)	L/L influence delayed colonic emptying	Camilleri, M, et al. (76)
IL10 (-1082)	Out-patient clinic, Rome I IBS	Association	230 IBS 450 Healthy controls	1.17 (0.93, 1.49)	Gonsalkorale, WM, et al. (216)
TGFB1 (*869* and *915*)	Out-patient clinic, Rome I IBS	Association	134 IBS 127 Healthy controls	NS	Gonsalkorale, WM, et al. (216)

*NS=not significant at p=0.05; IBD= inflammatory bowel disease; IBS= irritable bowel syndrome

Table 1.8: International population-based studies of the prevalence of MDD*.

			Prevalence				
Location	**MDD definition**	**Sample Size**	**Current**	**6-Month**	**1-year**	**Lifetime**	**Reference**
New Haven, CT	DIS→DSM-III	5,038		Men: 1.7 Women: 4.1	Men: 2.1 Women: 4.9	Men: 3.8 Women: 8.2	Weissman, MM (120)
USA (Epidemiologic Catchment Area)	DIS→DSM-III	18,572		2.2	3.0**	5.2**	Bland, RC (123) Weissman, MM (124)
Edmonton, Canada	DIS→DSM-III	~38,000 combined		3.2	5.2**	9.6**	Bland, RC (123) Weissman, MM (124)
Puerto Rico	DIS→DSM-III			3.0	3.0**	4.3**	Bland, RC (123) Weissman, MM (124)
Paris, France	DIS				4.5**	16.4**	Bland, RC (123) Weissman, MM (124)
Florence, Italy	DIS				5.2	12.4**	Bland, RC (123) Weissman, MM (124)
Beirut, Lebanon	DIS					19.0**	Bland, RC (123) Weissman, MM (124)
Seoul, Korea	DIS				2.3**	2.9**	Bland, RC (123) Weissman, MM (124)
Taiwan	DIS				0.8**	1.5**	Bland, RC (123) Weissman, MM (124)
New Zealand	DIS			5.3	5.8**	11.6**	Bland, RC (123) Weissman, MM (124)

Table 1.8 (Continued): International population-based studies of the prevalence of MDD*.

			Prevalence				
Location	**MDD definition**	**Sample Size**	**Current**	**6-Month**	**1-year**	**Lifetime**	**Reference**
USA (National Comorbidity Study)	CIDI				10.3	17.1	Bland, RC (123)
Ontario, Canada	CIDI, DSM-III-R				4.1 Men: 2.8 Women: 5.4		Bland, RC (123)
Britain (National Survey of Psychiatric Morbidity)	Clinical Interview Schedule→ICD-10	10,108	Age 16-54 Men: 1.7 Women: 2.7 Age 55-64 Men: 2.0 Women: 1.1				Bebbington, PE (96)
USA (National Comorbidity Survey)	CIDI→DSM-III-R	1,769	5.8		12.4	15.3	Kessler, RC (217)
USA (National Comorbidity Survey-Replication)	CIDI→DSM-IV	9,090			6.6	16.2	Kessler, RC (111)
Australia (Australian National Survey of Mental Health and Well-Being)	CIDI	10,641	3.2				Wilhelm, K (126)
Canada	CIDI	501			10.4		Patten, SB (125)

* DIS=Diagnostic Interview Schedule; DSM-III=Diagnostic and Statistical Manual, third edition; CIDI=Composite International Diagnostic Interview; DSM-III-R=Diagnostic and Statistical Manual, third edition, revised; ICD-10=International Classification of Diseases, tenth edition; DSM-IV=Diagnostic and Statistical Manual, fourth edition

** Rates standardized to the US population, ages 18-65

Table 1.9: Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Tsuang MT, et al. (218)	Admissions to University of Iowa Psychiatric Hospital between 1934 and 1944	Probands: 200 Schizophrenics 100 Manics 225 Depressives Relatives: 1578 Total first degree relatives 467 First degree relatives of depressives	Iowa Structured Psychiatric Interview Form (personal interview)	MDD prevalence: 8.8% among first degree relatives of MDD probands 4.6% among control probands Morbidity risk of MDD: 12.0 $\pm$ 1.76 among first degree relatives of MDD probands 7.3 $\pm$ 1.40 among control probands
Gershon ES, et al. (219)	Patients admitted for treatment of affective disorders at the National Institute of Mental Health Controls were admitted to medical institutes of National Institute of Health (psychiatrically normal)	172 Probands (30 MDD probands, 43 Control probands) 1254 relatives of probands and controls	Schedule of Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) and Research Diagnostic Criteria (RDC)	Lifetime prevalence of major affective disorder: 20% in relatives of MDD proband 7% in normal controls Morbidity risk of major affective disorder: 74% when both parents affected 27% when only one parent affected In controls, prevalence of major affective disorder in relatives lower than in proband relatives

Table 1.9 (Continued): Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Weissman MM, et al. (220)	Community sample in New Haven, CT Depressed probands received treatment at Yale outpatient between 1968 and 1977	335 Probands (163 MDD probands) 82 Normal controls 2003 First degree relatives	Modified SADS-L and RDC	Age-adjusted rate of MDD in first degree relative: 17.2-18.4/100 among mild and severe MDD probands 5.9/100 among normal controls Odds ratios of MDD: 2.48 for severe MDD vs normal controls 2.71 for mild MDD vs normal controls MDD aggregated in relatives of probands with mild and severe MDD
Maier W, et al. (221)	Psychiatric inpatients at hospital of University of Mainz, Germany Controls were people in Mainz with at least one accessible first degree relative	525 Probands (184 MDD probands) 109 General population control probands 514 Interviewed relatives of MDD probands 320 Interviewed general population control relatives	SADS-L, RDC, and some medical records	Prevalence of MDD: 11.7% in relatives of MDD probands 7.2% in normal controls 9.0% in alcoholic controls Lifetime morbidity risk of MDD: 21.6% in MDD proband relatives 10.6% in control relatives 12.0% in alcoholic control relatives

Table 1.9 (Continued): Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Weissman MM, et al. (222)	Cases: specialty clinics at Yale University; the Connecticut Mental Health Center, New Haven (Anxiety Research Clinic and the Depression Research Unit); and the New Haven site of the Epidemiologic Catchment Area (ECA) study Controls: ECA study controls	193 Probands (148 MDD, 45 control) 1047 Adult relatives	SADS-L and RDC	Aggregation of early-onset MDD, 21%, in relatives of probands with early-onset MDD Probands with early-onset MDD had 5 fold increased risk of early-onset MDD (OR 5.29, 95% CI 2.7-10.4) Early-onset MDD is not familialy associated with late-onset MDD
Sullivan PF, et al. (159)	Meta-analysis	5 previously reported studies		OR for proband 2.84 (95% CI 2.31-3.49)
Weissman MM, et al. (160)	Probands' parents from the Yale Family Study of Major Depression	220 children from 91 families: 125 children of 56 depressed probands and 95 children of 35 normal probands	SADS-L and RDC Children: K-SADS-E	Children of depressed probands reported more depression (RR 1.6, $p<0.05$) Children of depressed probands had a lower mean age of onset of depression (12.7 years) than children of normal probands (16.8 years), $p<0.001$

Table 1.9 (Continued): Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Hammen C, et al. (161)	Australian birth cohort of children born between 1981 and 1984 at the Mater Misericordiae Mother's Hospital in Brisbane, Queensland	816 mothers and their 15-year old adolescent children	Mothers: SCID Children: K-SADS-E	Children of mothers with major depression by age 10 were significantly more likely to have experienced major depression (χ^2=14.05, p<0.001, 20% depressed mothers and 10% non-depressed mothers) Children of mothers with only one major depressive episode had significantly higher rates of diagnosed depression (19% vs 10%; χ^2=6.83, p<0.001)
Lieb R, et al. (162)	Early Development Stages of Psychopathology Study (EDSP); 14-24 years at baseline; community; Germany	2427 respondents who completed the whole study period and for whom diagnostic information about psychopathology in both parents was available	Offspring: M-CIDI/DSM-IV Parental History: M-CIDI family history module	Offspring of either 1 or 2 depressed parents reported higher rates of depression (26.1% and 28.5% versus 12.3%) Parental major depression increases offspring risk of depression

Table 1.9 (Continued): Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Klein DN, et al. (164)	Oregon Adolescent Depression Project family study; community sample of adolescents	30 probands with lifetime history of dysthymic disorder 65 probands with history of chronic MDD 313 probands with history of episodic MDD 392 probands with no lifetime history of any mood disorders 2750 First-degree relatives	Adolescents: Schedule for Affective Disorders and Schizophrenia for School-Age Children, with additional items to derive DSM-III-R diagnoses Relatives: SCID, non-patient version; family history by the Family Informant Schedule and Criteria, modified for DSM-IV diagnoses	Relatives of chronic MDD probands had a higher rate of MDD than either relatives of episodic MDD probands or relatives of probands with no history of mood disorders (HR=1.41, 95% CI=1.07-1.87, p<0.02; HR=2.08, 95% CI=1.56-2.76, p<0.001, respectively) Attenuation of rate of MDD when comparing relatives of episodic MDD probands to probands with no history of mood disorders (HR=1.47, 95% CI=1.21-1.79, p<0.001) Similar relationships existed when examing the rate of recurrent MDD Rate of chronic MDD was greater in the relatives of chronic MDD probands than in the relatives of probands with no history of mood disorders (HR=2.30, 95% CI=1.26-4.20, p<0.01) Higher rates of episodic MDD were found among relatives of chronic MDD probands (HR=1.91, 95% CI=1.38-2.65, p<0.001) and among relatives of epidsodic MDD probands (HR=1.46, 95% CI=1.17-1.81, p<0.001), compared with relatives of probands with no history of mood disorders

Table 1.9 (Continued): Family Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Klein DN, et al. (163)	Oregon Adolescent Depression Project family study; community sample of adolescents	268 adolescents with MDD 110 adolescents with a history of non-mood disorders but no history of MDD 291 adolescents with no history of psychopathology through age 18 2202 first-degree relatives	Adolescents: Schedule for Affective Disorders and Schizophrenia for School-Age Children, with additional items to derive DSM-III-R diagnoses Relatives: SCID, non-patient version; family history by the Family Informant Schedule and Criteria, modified for DSM-IV diagnoses	Rate of MDD was significantly elevated in the relatives of adolescent probands with a history of MDD (HR=1.77; 95% CI 1.46-2.31) Rate of MDD in relatives did not differ as a function of sex of the proband

* MDD= major depressive disorder; SADS-L=schedule of affective disorders and schizophrenia-lifetime version; RDC=research diagnostic criteria; OR=odds ratio; 95% CI= 95% confidence interval; RR= risk ratio; M-CIDI=maternal-composite international diagnostic interview; DSM-IV= Diagnostic and Statistical Manual, fourth edition; HR=hazard ratio; SCID=structured clinical interview for DSM-IV; DSM-III-R= Diagnostic and Statistical Manual, third edition, revised

Table 1.10: Adoption Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Von Knorring AL, et al. (165)	Stockholm Adoption Study (adoptive and biologic mothers and fathers of adoptees with affective disorders and histories of substance abuse)	115 adoptees with disorder 115 control adoptees biologic and adoptive mothers and fathers	Health insurance: F-card psychiatric illness, defined by being on the National Health Insurance Board sick list at least 2 weeks with psychiatric diagnosis (ICD-8, codes 290-309) and treated as outpatients or inpatients in a psychiatric department or mental hospital	Risk of affective disorder: 6.1% in biologic mothers 2.5% in biologic fathers 3.0% in both adoptive mothers and fathers No evidence of familial aggregation of parents and adoptees with same diagnosis among biologic relatives Concordance for psychiatric treatment among adoptive fathers and their children Psychiatric patients had a 5-fold excess of adoptive fathers with psychiatric illness compared to fathers of matched controls Biologic mothers of female patients had a 3-fold increase in psychiatric illness compared to mothers of matched controls
Wender PH, et al. (167)	Denmark adoptees Cases: adoptees with affective disorders Controls: demographically matched adoptees without known mental illness	71 cases (27 with MDD) 71 controls	Cases: discharge diagnoses from psychiatric facility (taken from Psychiatric Register and the Register of the Bispebjerg Hospital) Relatives: DSM-III as taken from death and psychiatric records	Biologic relatives had greater frequency of same affective disorders as the index case (p=0.03) No difference in frequency of MDD among index and control among adoptive relatives

Table 1.10 (Continued): Adoption Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Cadoret RJ, et al. (166)	Adoptees from two agencies in Iowa: Iowa Children's and Family Services and Lutheran Social Services (both in Des Moines, IA)	443 adoptees (48 with MDD)	DSM-III major depression (SADS-L, DIS, or earlier interview based on Feighner criteria)	Exposure to environmental factors before age 18 of the adoptees predisposed them to depression Correlation of MDD with a biologic background of affective disorders: Men: 15% of those with MDD had a biologic relative with affective disorder 7% of those with MDD did not have a biologic relative with affective disorder Women: 28.6% of those with MDD have a biologic relative with an affective disorder 13.9% of those without MDD did not have a biologic relative with an affective disorder

*ICD-8=International Classification of Diseases, 8th edition; MDD=major depressive disorder; DSM-III= Diagnostic and Statistical Manual, third edition; SADS-L=schedule of affective disorders and schizophrenia-lifetime version; DIS=diagnostic interview schedule

Table 1.11: Twin Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
McGuffin P, et al. (223)	Probands ascertained via Maudsley Hospital Twin Register (London, England)	177 MDD probands and their co-twins	Diagnosis on twin registry DSM-III-R Present State Exam (PSE)	MDD concordance: MZ 46% (n=68) DZ 20% (n=109) Heritability of MDD: Between 48-75% based on varying baseline prevalence of MDD
Kendler KS, et al. (224)	Psychiatric hospitalization from Swedish Psychiatric Twin Registry	1002 twin probands	DSM-III-R	MDD concordance: MZ men: 50% DZ men: 33% MZ women: 32% DZ women: 20% (159)
Lyons MJ, et al. (225)	Male twins Vietnam Era Twin Registry	3372 twin pairs (1874 MZ pairs and 1498 DZ pairs)	Standardized telephone interview, DIS version III Revised DSM-III-R	9.2% MDD prevalence MDD concordance: MZ 22.5% DZ 14.0% 36% heritability of MDD 64% of variance in liability due to unique environment
Kendler KS, et al. (224)	General population Swedish Twin Registry	800 twin pairs	DSM-III-R	MDD concordance: MZ men: 40% DZ men: 33% MZ women: 67% DZ women: 32% (159)

Table 1.11 (Continued): Twin Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Bierut LJ, et al. (226)	Australian community based twin sample	2662 twin pairs (women=3494, men=1830)	Semi-structured assessment for the Genetics of Alcoholism (semi-structured lay interview designed to assess psychiatric symptoms) DSM-III-R DSM-IV Severe DSM-IV MDD	DSM-III-R Lifetime MDD prevalence: Men: 24% Women: 31% MDD concordance: MZ men: 34% DZ men: 30% MZ women: 50% DZ women: 37% MDD heritability: Men: 24% Women: 44% DSM-IV Lifetime MDD prevalence: Men: 16% Women: 22% MDD concordance: MZ men: 20% DZ men: 23% MZ women: 38% DZ women: 25% MDD heritability: Men: 18% Women: 36%

Table 1.11 (Continued): Twin Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Kendler KS and Prescott CA (227) Erratum (228)	Virginia Twin Registry	3790 complete twin pairs	Telephone interview Lifetime history MDD DSM-III R (SCID-based)	MDD concordance: MZ men: 41% DZ men: 34% MZ women: 47% DZ women: 43% Male-male twin pairs: OR MZ: 3.29 OR DZ: 1.86 MDD heritability: 38% Unique environment: 62% Female-female twin pairs: OR MZ: 3.02 OR DZ: 1.59 MDD heritability: 39% Unique environment: 61% All types of twin pairs: MDD heritability: 39% (95% CI: 30-47%) Unique environment: 61%

Table 1.11 (Continued): Twin Studies of Major Depressive Disorder*.

Author	Population	Sample size	MDD definition	Results
Sullivan PF, et al. (159)	Meta-analysis; clinic and community samples	21,000 twin pairs	DSM-III-R	Overall: Best model was genetic and unique environment Heritability: 37% (95% CI 33-42%) Unique environment, 63% (95% CI 58-67%) Community studies Heritability: 37% (95% CI 28-42%) Clinic studies Heritability: 43% (95% CI 21-58%)
Fu Q, et al. (168)	Vietnam Era Twin Registry	3360 male-male twin pairs	Computerized telephone version of the DIS, version 3, revised (DSM-III-R)	Lifetime prevalence of DSM-III-R MDD was 9.2% Heritability of lifetime major depression was 40%
Kendler KS and Aggen SH (169)	Virginia Twin Registry	858 female-female twin pairs	Structured psychiatric interview based on the SCID (DSM-III-R)	Heritability estimates of MDD between 0.34 and 0.41 for the four time periods of interviews

*MDD=major depressive disorder; PSE=present state exam; DSM-III-R=Diagnostic and Statistical Manual, third edition, revised; MZ=monozygotic; DZ=dizygotic; DIS=diagnostic interview schedule; DSM-IV=Diagnostic and Statistical Manual, fourth edition; SCID=structured clinical interview for DSM-IV; OR=odds ratio; 95% CI= 95% confidence interval

Table 1.12: Linkage Studies in Major Depression*.

Gene (Polymorphism)	Population	Size	Results	Reference
27 markers on Chromosomes 16, 18, 21, and 4p	Swedish Recurrent MDD	5 families defined by recurrent MDD proband—60 individuals; 19 recurrent MDD	No significant linkage found	Balciuniene J, et al. 1998 (229)
451 Kb region of 2q33-34 between markers D2S2321 and D2S2208	Proband defined as recurrent early-onset MDD (SADS-L)	81 families (all affected relative pairs)—407 first degree relatives, 835 extended relatives	Men—Not significant Women—Similar LOD scores as sex-dependent reported below **Multi-point Linkage** Major Mood Disorder D2S2321 LOD 4.592 D2S2208 LOD 4.753 Major or Minor Mood Disorder D2S2321 LOD 6.331 D2S2208 LOD 6.866 Recurrent Early Onset Major Depression D2S2321 LOD 3.108 D2S2208 LOD 3.167 Recurrent Major Depression D2S2321 LOD 3.229 D2S2208 LOD 3.314	Zubenko GS, et al. 2002 (171)

Table 1.12 (Continued): Linkage Studies in Major Depression*.

Gene (Polymorphism)	Population	Size	Results	Reference
Genomewide scan with 628 microsatelite markers	Mormon population of Utah Pedigrees ascertained for MDD	110 Utah pedigrees 1890 individuals	Men MDD D12S1300 LOD 4.6, p=0.00003 D12S1706 LOD 6.1, p=0.0000007	Abkevich V, et al. 2003 (172)
38 SSTRPs covering 12 chromosomal regions with genes involved in neuroendocrine regulation or serotonergic neurotransmission	US Recurrent, early-onset (≤25 yo) major depression SADS-L	34 families in total 16 families (initial), n=148 18 additional families, n=97	Using pairwise linkage analysis, none of the markers were significant for major depression	Neiswanger K, et al. 1998 (117)
D2S2944, 124-bp allele	US Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID	100 RE-MDD 100 controls	Men: no significant effect on RE-MDD Women: evidence of linkage and linkage disequilibrium of allele with RE-MDD (χ^2=5.23, p=0.02)	Zubenko GS, et al. 2002 (170)

Table 1.12 (Continued): Linkage Studies in Major Depression*.

Gene (Polymorphism)	Population	Size	Results	Reference
Genome-wide linkage using 392 highly informative polymorphisms spaced 9cM apart on average	US Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID	81 families identified by proband with recurrent early onset major depressive disorder (407 1st degree relatives and 835 extended relatives)	2 regions demonstrated significant linkage for RE-MDD and R-MDD: 11pter-p15, marker D11S1984, multipoint LOD 4.20, p<0.0001; and 11q13-14, marker D11S2002, multipoint LOD 2.51, p<0.0001	Zubenko GS, et al. 2003 (173) Early-onset <=25 years
Genome scan using 389 microsatellite markers (mean spacing of 9.3 cM)	Six sites across the US involved in the Genetics of Recurrent Early-Onset Depression (GenRED) project Opportunistic recruitment/ascertainment Interviews—DIGS and FIGS DSM IV	297 families including 685 informative affected relative pairs	Chromosome 15q25.3-26.2 had a significant peak, LOD=3.73, p=0.23 This peak mapped to the 103.4 cM of the decode map, flanked by the D15S652 and D15S816 markers	Holmans P, et al. 2004 (174) Early-onset was before age 31 for probands or age 41 for other affected relatives

Table 1.12 (Continued): Linkage Studies in Major Depression*.

Gene (Polymorphism)	Population	Size	Results	Reference
Alpha2 adrenergic receptors	Univerisity of Iowa Psychiatric Hospital or the Veterans Administration Hospital in Iowa City Feighner criteria	17 pedigrees, 7 with pure depressive disease and 10 with depression spectrum disease involved 161 individuals, 49 with unipolar depression	All analyses had LOD scores less than -2, indicating linkage exclusion for these candidate genes	Wang Z, et al. 1992 (230)

* MDD=Major Depressive Disorder; SADS-L=Schedule of Affective Disorders and Schizophrenia—Lifetime version; LOD=Log Odds; SSTRPs=Single Sequence Tandem Repeat Polymorphisms; SCID=Structured Clinical Interview for Diagnostic and Statistical Manual, 3rd edition

Table 1.13: Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
SLC6A4 (VNTR and I/D)		Meta-analysis	*VNTR*: 299 *I/D*: 275	*VNTR*: 772 *I/D*: 739	No significant associations of VNTR and unipolar patients Significant association with promoter allele 2 and risk of unipolar disorder (OR 1.23, 95% CI 1.01-1.52, χ^2=4.10, p=0.042)	Furlong RA, et al. 1998 (181)
SLC6A4 (*VNTR*)	Han Chinese	MDD patients	33	362	Allele 12 associated with MDD (p=0.0107) 12/12 homozygous genotype associated with MDD (p=0.0137)	Liu N, et al. 1999 (180)
SLC6A4 (VNTR)	UK	DSM-IV Lithium clinic out-patients	86	187	NS	Collier DA, et al. 1996 (175) No assessment of control mental health
SLC6A4(VNTR)	Japanese	RDC	42	137	NS	Kunugi H, et al. 1996 (199)
SLC6A4 (VNTR)	Germany	DSM-IV SADS-L Recurrent MDD patients	49	218	NS	Stober G, et al. 1996 (197)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
SLC6A4 (VNTR)	English and Scottish	DSM-IV SADS-L MDD in- and out-patients Controls SADS-L for mental health	39	123 controls (not screened psych disorders) 71 controls screened with SADS-L	Two control groups had similar frequencies of alleles, and thus were pooled into one control group Significant genotype distribution difference between MDE patients and controls (χ^2=10.05, p<0.004 (Bonferroni corrected), OR 6.95 (1.8-27.2))—patients had higher frequency of being homo or heterozygous for the 9 repeat allele Significant allele frequency difference between MDE patients and controls (χ^2=9.87, p<0.02 (Bonferroni corrected), OR 6.51 (1.7-24.9))—unipolar patients had a higher frequency of 9 repeat allele than controls	Ogilvie AD, et al. 1995 (179)
SLC6A4 (VNTR and I/D)	France and Germany	DSM-IV SADS-L MDD out-patients	36	294	NS	Hoehe MR, et al. 1998 (200)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
SLC6A4	Postmortem suicides	DSM-III-R by psychological autopsy	82	138	NS allele frequency distribution Genotype frequency varied, those with MDD had higher rate of heterozygotes (χ^2=9.6, p=0.008)	Mann JJ, et al. 2000 (176)
SLC6A4 (VNTR and I/D)	UK	DSM-IV SCAN interview MDD in- and out-patients	80 (lifetime MDD)	121	*VNTR*: genotype and allele frequencies not different cases and controls; however there is a difference when examining those not taking lithium and controls, p=0.041 for allele 9 presence *I/D*: no allele frequency difference statistically significant; those taking lithium had a sig decrease in frequency of the deleted allele compared to controls (χ^2=6.71, p=0.010)	Rees M, et al. 1997 (177) Controls not screened for psych disorders
SLC6A4 (VNTR and I/D)	UK	SADS-L, case note MDD in- and out-patients	125	174	NS	Furlong RA, et al. 1998 (181)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
SLC6A4 (VNTR and I/D)	Japanese	DSM-IV MDD in- and out-patients Controls were medical students and hospital staff	49	212	*VNTR*: NS *I/D*: NS	Kunugi H, et al. 1997 (198) Controls did not undergo psych evaluation
SLC6A4 (VNTR and 5-HTTLPR)	Spanish	Personal interview MDD out-patients	74	84	*VNTR*: NS *5-HTTLPR*: NS Overall distribution of haplotypes differed significantly with the 10 repeat allele having increased risk for disease (χ^2=7.298, p=0.0069, OR=2.53, 95% CI 1.21-5.34)	Gutierrez B, et al. 1998 (201)
SCL6A4 (VNTR and 44-BP Ins/Del)	Denmark	MDD in-patients	92	108	NS	Mellerup E, et al. 2001 (202)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
SLC6A4 (I/D)	Polish	SCID MDD in-patients	94	213	Everyone: statistically significant difference in allele and genotype frequencies between unipolar cases and controls (p=0.001; χ^2=11.760, p=0.003, respectively) Males: statistically significant difference in allele frequency between cases and controls (p=0.031) (unipolar had higher frequency of short allele), but not in genotype frequency (χ^2=5.283, p=0.071) Females: NS	Hauser J, et al. 2003 (178) Controls not psychiatrically screened
SLC6A4 (I/D)	Italy	OPCRIT and/or SADS-L MDD in-patients	67	0	NS	Serretti A, et al. 1999 (231)
HTR2A (T102C) *HTR2A (His452Tyr)* *SLC6A4*	Germany	DSM-IV	137	121	NS	Minov C, et al. 2001 (232)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
HTR2A (T102C)	Spanish	SCID MDD out-patients	159	162	No overall differences in genotype or allele frequencies between cases and controls Differences existed when MDD separated into seasonal and non-seasonal for the genotype (χ^2=10.63, p=0.004)	Arias B, et al. 2001 (193)
HTR2A (T102C)	European (ECPAD)		142	142	NS	Oswald P, et al. 2003 (233)
HTR1B (G861C)		SCID	208	183 inpatients without MDD 96 healthy	Significant differences in genotype and allele frequencies between those patients with MDD and those without MDD (χ^2=6.83, p=0.033; χ^2=5.81, p=0.016, respectively) MDD associated with *HTR1B G861C* locus, OR=0.46, 95% CI=0.31-0.68, χ^2=14.93, p=0.001, independent of race MDD associated with *HTR1B G861C* locus, OR=1.64, 95% CI=1.16-2.31, χ^2=7.81, p=0.005, independent of sex	Huang Y, et al. 2003 (185)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
HTR5A (-19G/C and *12A/T*)	Spanish	SCID	181	157	NS	Arias B, et al. 2001 (234)
HTR6 (*C267T*)	China	DSM-IV	77	147	NS	Hong CJ, et al. 1999 (150)
HTR2C (*cys23ser*)	European (ECPAD)	European Collaborative Project on Affective Disorders (ECPAD)	513 Recurrent	901	MDD associated with *ser23* allele carrier status (χ^2=7.34, p=0.006)	Lerer B, et al. 2001 (115)
TPH1 *HTR2A* *HTR2C* *SLC6A4* *DRD4* *DAT1* *COMT*	Ashkenazi and non-Ashkenazi Jews	SADS-L or SCID	102	172	NS	Frisch A, et al. 1999 (235)
DRD2 (*-141CIns/Del*)	UK caucasians	MDD patients	128	262	NS	Furlong RA, et al. 1998 (236)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
DRD2 (Ser^{311}/Cys^{311}) *DRD3 (Ser^{9}/Gly^{9})* *DRD4* *DAT1*	Japanese	DSM-III-R Hospital employee and their friends as controls	49	100	*DRD2*, *DRD3*, and *DAT1* not significant *DRD4*: Frequency of 4 and 5 repeat allele significantly different between patients (4-allele χ^2=6.49, p=0.011; 5-allele χ^2=4.18, p=0.041) and controls, but frequency of 2 repeat allele not significant 4-allele less frequent in MDD patients 5-allele more frequent in MDD patients 4-allele homozygotes significantly less frequent in MDD (58.2%) than controls (80%), χ^2=7.26, p=0.007 4/5-allele heterozygotes significantly more frequent in MDD (18.4%) than controls (7%), χ^2=4.43, p=0.035	Manki H, et al. 1996 (184)
DRD2 *DRD3*	European (ECPAD)		*DRD2*: 133 *DRD3*: 136	*DRD2*: 133 *DRD3*: 136	NS	Massat I, et al. 2002 (237)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
DRD4	Italy	MDD in-patients	126	471	Exon 1 and 3 polymorphisms did not differ in allele or genotype distribution Trend observed toward excess of *DRD4*2/4* (χ^2=8.34, df=1, p=0.004 OR=2.09; 95% CI= 1.27-3.43)	Serretti A, et al. 1999 (238) Power low but higher than 60%
DRD4 *TH*	Croatia	SADS-L, clinical interviews, hospital case notes	41	71	NS	Oruc L, et al. 1997 (239)
TH (tetranucleotide repeat polymorphism (TH4) in intron 1 and the 5' TaqI RFLP polymorphism)	Belgian	SADS-LA	35 (at least 2 MDE)	50	*TH4* polymorphism: NS 5'TaqI polymorphism: significant genotype frequency differences between cases and controls (χ^2=9.99, p=0.002, after correcting for multiple comparisons, p=0.006) the 2-2 genotype was significantly more often observed in unipolar patients	Souery D, et al. 1996 (192)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
TH (tetranucleotide repeat)	UK	MDD out-patients	126	242	*TH* tetranucleotide repeat: No significant differences in allele or genotype frequencies (χ^2=4.33, df=4, p=0.36; χ^2=17.69, df=14, p=0.22, respectively) *PstI polymorphism:* Cambridge sample demonstrated a significant difference in allele frequency between cases and controls (χ^2=3.946, df=1, p=0.047); no such difference for Edinburgh samples No difference in genotype frequencies between cases and controls in either Cambridge or Edinburgh Significant test of association (p<0.05), estimated OR 0.72 (95% CI 0.54-0.97) for allele B vs allele A, and 0.55 (95% CI 0.25-0.993) for genotype BB vs genotype AA	Furlong RA, et al. 1999 (191)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
MAOA (*MAOA-LPR*)	Germany	DSM-IV Controls from blood donor center	74 recurrent	229	NS	Syagailo YV, et al. 2001 (240)
MAOA	Germany	In-patients	146 patients (42 MDE and 104 recurrent MDD)	101	Significant genotype frequency differences among women with recurrent MDD and controls (χ^2=4.767, p=0.029) Trend of the longer allele being more frequent in controls (p=0.053)	Schulze TG, et al. 2000 (116)
MAOA (*T941G*)	Germany	MDD out-patients	108	276	NS	Tadic A, et al. 2003 (136)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
MAOA (*MAOA-LPR*)	Germany	DSM-IV Controls from blood donor center	74 recurrent	229	NS	Syagailo YV, et al. 2001 (240)
TPH1 (218 A→C, -1067 G→A, and -347 T→G)	Chinese	DSM-IV MDD out-patients	91	139	No significant difference in genotype or allele frequency between cases and controls for -1067 G→A or -347 T→G Significant genotype frequency difference between cases and controls for 218 A→C polymorphism (χ^2=6.915, p=0.032)	Tan EC, et al. 2003 (183)
TPH1 (*A281C*)	UK	MDD out-patients	125	437	NS	Furlong RA, et al. 1998 (241) 80% power to detect gene with a RR of 1.5-2.0
CLOCK (*T3111C*)	European Americans	DSM-IV/SCID	143	195	NS	Desan PH, et al. 2000 (242)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
TPH2 (10 SNPs in the region)	Germany	MDD in-patients	300	265	Significant allele frequency difference for SNP rs1386494 (A/G poly), cases had higher G frequency than controls (χ^2=10.51, p=0.0012; OR=0.60; Bonferroni corrected p=0.012) Marginally significant allele frequency difference for SNP rs1843809 (G/T poly) (χ^2=3.85, p=0.0496; OR=1.38; Bonferroni correction was not significant) Three haplotypes significantly differed between cases and controls, p<0.00001; one more frequent in controls and two only present in cases	Zill P, et al. 2004 (189)
NET (*T-182C*)	Korea	DSM-IV	112	136	NS	Ryu SH, et al. 2004 (243)
NET (*T-182C* and *G1287A*)	Germany	MDD patients	193	136	NS	Zill P, et al. 2002 (134)
NET (*G1287A*)	Canada	Recurrent MDD DSM-IV	105	74	NS	Owen D, et al. 1999 (142)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
CYP2C9 (**1*, **2*, **3*)	Spanish	DSM-IV	70 MDD	89 Schizophrenic 138 healthy	No differences in *CYP2C9*1* or **2* allele *CYP2C9*3* allele frequency higher in MDD patients than in healthy controls ($p<0.01$) or schizophrenia controls ($p<0.01$) OR *CYP2C9*3* allele: 2.76 (1.51-5.05) in MDD vs healthy controls 3.30 (1.5-6.13) in MDD vs schizophrenia controls	Lerena A, et al. 2003 (186)
CRHR2 (*CRHR2s03, CRHR2s04, CRHR2s181, CRHR2s183, CRHR2s185*)	Belgian	MDD in-patients	89	89 age-, gender-, and ethnicity-matched controls	Significant genotype frequency between cases and controls for *CRHR2s183* ($p=0.03$) Significant allele frequency difference between cases and controls for *CRHR2s183*, with the A allele being more common in cases than controls (12% vs 6%, $p=0.04$) Other markers not significant	Villafuerte SM, et al. 2002 (188)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
CCK (*STR*)	Japanese	MDD in- and out-patients	82	253	NS	Hattori E, et al. 2002 (244)
GABRA3 (CA-repeat)	Germany	MDD in-patients	201	151	Males: NS Females: No significant genotype differences between cases and controls for alleles 1 and 4 (χ^2=9.66, df=6, p=0.13; χ^2=7.75, df=1, p=0.08, respectively) Significant allele distribution difference between cases and controls for alleles 1 and 4 (χ^2=13.21, df=3, p≤0.0001; χ^2=12.66, df=1, p≤0.0001, respectively)	Henkel V, et al. 2004 (190)
ACE I/D	Germany	MDD in-patients	63	169	NS	Pauls J, et al. 2000 (245)
ACE I/D	UK	MDD in-patients	169	313	NS	Furlong RA, et al. 2000 (246)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
ACE (I/D)	Japan	DSM-III-R Clinical interview and medical records MDD in-patients	34	579	NS	Arinami T, et al. 1996 (247)
GNAL	Germany	ICD-10 DSM-IV	176	145	No significant differences in allele or genotype frequencies for either polymorphism between cases and controls Intron 3: allele, p=0.339 Genotype, χ^2=1.813, p=0.404 Intron 10: allele, p=0.352 Genotype, χ^2=1.069, p=0.586 Gender effect in intron 3: females stat sig higher g-allele frequencies than males, p=0.0036; genotype, χ^2=9.069, p=0.011	Zill P, et al. 2002 (194)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
PLA2	Belgium, Bulgaria, Croatia, Germany, Greece, Italy (BIOMED 1 and BIOMED 2)	DSM-IV DSM-III-R RDC	328	630	After controlling for population group and gender, allele 7 had a stat sig association among unipolar depressives with more than three MDEs, OR 1.40, 95% CI 1.09-1.81, p=0.01 compared to controls	Papadimitriou GN, et al. 2003 (182)
DDC (1-bp deletion and 4-bp deletion)	Germany	DSM-IV SADS-L, family history, OPCRIT, medical records MDD in-patients	183	234	NS	Jahnes E, et al. 2002 (248)
CTLA4 (*A49G*)	Korean	DSM-IV	77	149	NS	Jun TY, et al. 2001 (249)
WFS1 (His611Arg)	UK	RDC SADS-L, case note review MDD in- and out-patients	163	316	NS	Furlong RA, et al. 1999 (250)

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
10 cM systematic survey of human genome using SSTRPs	US	Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID-I-NP and SCID-II	100 RE-MDD	100 healthy controls, matched by age, sex, race, and ethnicity	Significant allele differences in 19 SSTRPs, 9 specific for women, 7 specific for men, and 3 sex-independent **Female specific** D1S1677 (χ^2=5.78, p=0.016) D2S2944 116 bp (χ^2=5.64, p=0.018) 124 bp (χ^2=13.06, p=0.0003) D5S1462 217 bp (χ^2=7.79, p=0.005) 229 bp (χ^2=8.41, p=0.004) D13S317 175 bp (χ^2=6.82, p=0.009) 195 bp (χ^2=6.82, p=0.009) ACTC 233 bp (χ^2=5.18, p=0.023) 237 bp (χ^2=7.79, p=0.005) 241 bp (χ^2=5.60, p=0.018)	Zubenko GS, et al. 2002 (251) Sex-specificity of susceptibility loci for RE-MDD Both protective and increased risk is associated with these

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
10 cM systematic survey of human genome using SSTRPs	US	Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID-I-NP and SCID-II	100 RE-MDD	100 healthy controls, matched by age, sex, race, and ethnicity	**Female specific (continued)** D18S858 (χ^2=7.68, p=0.006) D20S478 (χ^2=11.05, p=0.001) GATA31E08 238 bp (χ^2=8.00, p=0.005) 246 bp (χ^2=4.57, p=0.033) DXS6800 197 bp (χ^2=5.63, p=0.018) 205 bp (χ^2=5.94, p=0.015)	Zubenko GS, et al. 2002 (251) Sex-specificity of susceptibility loci for RE-MDD Both protective and increased risk is associated with these

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
10 cM systematic survey of human genome using SSTRPs	US	Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID-I-NP and SCID-II	100 RE-MDD	100 healthy controls, matched by age, sex, race, and ethnicity	Significant allele differences in 19 SSTRPs, 9 specific for women, 7 specific for men, and 3 sex-independent **Male specific** D6S1056 (χ^2=4.71, p=0.030) D10S1248 (χ^2=8.56, p=0.003) D10S189 (χ^2=7.23, p=0.007) D15S643 207 bp (χ^2=6.11, p=0.013) 211 bp (χ^2=9.07, p=0.003) 223 bp (χ^2=5.84, p=0.016) D16S3253 (χ^2=9.51, p=0.002) D19S178 (exact p, p=0.035) D21S2055 117 bp (χ^2=6.66, p=0.010) 129 bp (χ^2=6.08, p=0.014) 153 bp (χ^2=4.39, p=0.036)	Zubenko GS, et al. 2002 (251) Sex-specificity of susceptibility loci for RE-MDD Both protective and increased risk is associated with these

Table 1.13 (Continued): Association Studies in Major Depression*.

Gene (Polymorphism)	Ethnicity	Diagnostic criteria /instrument	Cases	Controls	Results (gene/allele frequencies)	Reference
10 cM systematic survey of human genome using SSTRPs	US	Recurrent, early-onset (≤25 yo) major depression (SADS-L) Controls: SCID-I-NP and SCID-II	100 RE-MDD	100 healthy controls, matched by age, sex, race, and ethnicity	Significant allele differences in 19 SSTRPs, 9 specific for women, 7 specific for men, and 3 sex-independent **Sex-independent** D12S398 132 bp (χ^2=9.01, p=0.003) 140 bp (χ^2=7.09, p=0.008) D15S822 258 bp (χ^2=9.34, p=0.002) 294 bp (χ^2=5.85, p=0.016) D17S1293 262 bp (χ^2=7.11, p=0.008) 274 bp (χ^2=5.31, p=0.021) 286 bp (χ^2=4.63, p=0.031)	Zubenko GS, et al. 2002 (251) Sex-specificity of susceptibility loci for RE-MDD Both protective and increased risk is associated with these

*_SLC6A4_=Serotonin Transporter Protein; VNTR=Variable Number of Tandem Repeats; I/D=Insertion/Deletion; OR=Odds Ratio; 95% CI=95% Confidence Interval; MDD=Major Depressive Disorder; DSM-IV=Diagnostic and Statistical Manual, 4th edition; SADS-L=Schedule of Affective Disorders and Schizophrenia—Lifetime version; MDE=Major Depressive Episode; RDC=Research Diagnostic Criteria; DSM-III-R=Diagnostic and Statistical Manual, 3rd edition revised; _5-HTTLPR_=serotonin transporter long polymorphic repeat; OPCRIT=Operational Criteria Checklist; _HTR2A_=Serotonin Transporter Type 2A receptor; NS=not significant; SCID=Structured Clinical Interview for Diagnostic and Statistical Manual; ECPAD=European Collaborative Project on Affective Disorders; _HTR1B_=Serotonin Transporter Type 1B receptor; _HTR5A_=Serotonin Transporter Type 5A receptor; _HTR6_=Serotonin Transporter Type 6 receptor; _HTR2C_=Serotonin Transporter Type 2C receptor; _TPH1_=Tryptophan Hydroxylase; _DRD4_=Dopamine Receptor D4; _DAT1_=Dopamine Transporter Gene; _COMT_=Catechol-O-Methyl Transferase; _DRD2_=Dopamine Receptor D2; _DRD3_=Dopamine Receptor D3; _TH_=Tyrosine Hydroxylase; RFLP=Restriction Fragment Length Polymorphism; _MAOA_=Monoamine Oxidase A; _TPH2_=Tryptophan Hydroxylase 2; SNP= Single Nucleotide Polymorphism; _CLOCK_=Circadian Locomotor Output Cycles Kaput; _CYP2C9_=Cytochrome P450 2C9; _NET_=

Norepinephrine Transporter; *CRHR2*=Corticotropin-Releasing Hormone Receptor 2; *CCK(STR)*=Cholecystokinin (Short Tandem Repeat); *GABRA3*=Gamma-Aminobutyric Acid Receptor, alpha-3; *ACE*=Acetylcholine; *GNAL*=Guanine Nucleotide-binding Protein, alpha-activating activity polypeptide, olfactory type; ICD-10=International Classification of Disease, 10th edition; *PLA2*=Phospholipase A2; BIOMED1=European Collaborative Biomedical Research Project 1; BIOMED2=European Collaborative Biomedical Research Project 2; *DDC*=Dopa Decarboxylase; *CTLA4*=Cytotoxic T Lymphocyte-associated 4; *WFS1*=Wolfram syndrome (WFS) gene; SSTRP=Single Sequence Tandem Repeat Polymorphism; SCID-I-NP= Structured Clinical Interview for DSM-III, Axis I disorders, Non-patient; SCID-II=Structured Clinical Interview for DSM-III, Axis II Disorders; RE-MDD=Recurrent Major Depressive Disorder

Table 1.14: *SLC6A4 (VNTR)* results from Anguelova et al. (195)*.

	OR	95% CI	P-value
Allele 9	1.24	0.72-2.14	0.493
Allele 10	0.96	0.84-1.10	0.585
Allele 12	1.03	0.89-1.18	0.747

**SLC6A4 (VNTR)*=serotonin transporter protein (variable number of tandem repeats); OR=odds ratio; 95% CI=95% confidence interval

1.4 FIGURES

Figure 1.1: Tryptophan Metabolic Pathways. As demonstrated in this diagram, tryptophan is the precursor of serotonin (5-HT). The rate-limiting step in the synthesis of 5-HT is the conversion of tryptophan into 5-Hydroxytryptophan by the enzyme tryptophan hydroxylase. Additionally, the alternate pathway for tryptophan, the kynurenine pathway (right-side), is its major route of degradation in the brain and liver. In depressed individuals, an increase in kynurenine concentration and a subsequent decrease in serotonin concentration has been demonstrated (adapted from Struder (152)).

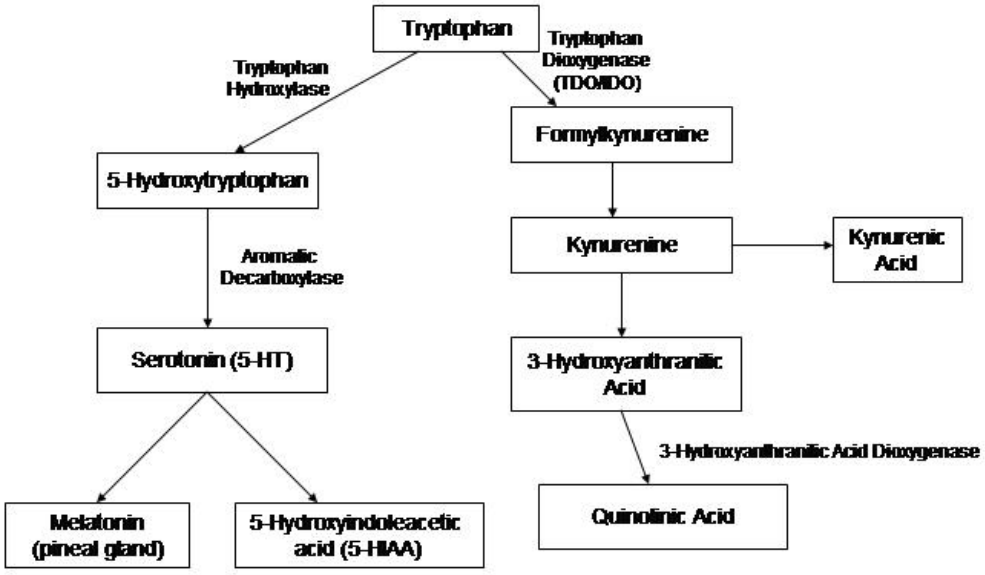
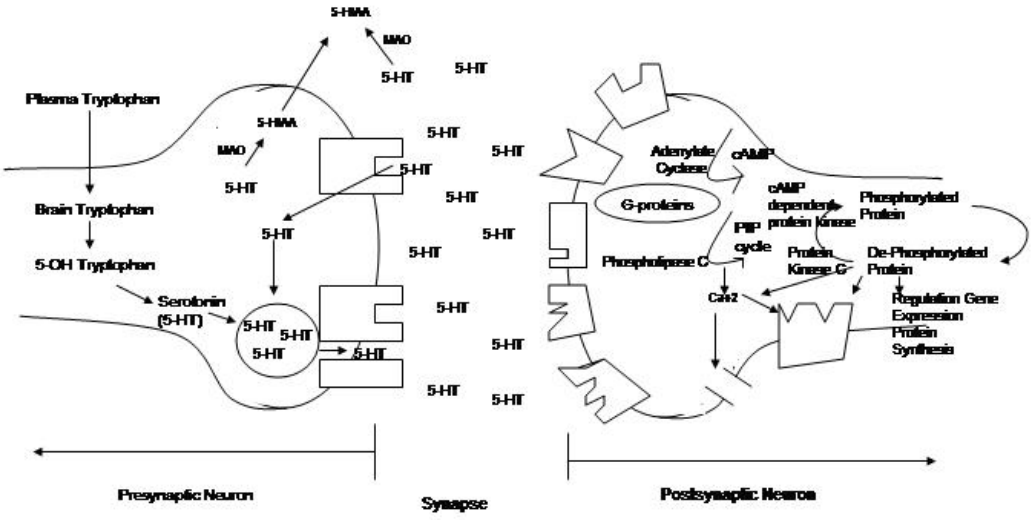


Figure 1.2: Schematic Drawing of a Serotonin Neuron Synapse. This diagram depicts a serotonin neuron synapse, which could easily be a norepinephrine synapse. In the presynaptic neuron, plasma tryptophan comes into the brain and is converted to serotonin which is then transported into the synapse when a signal (action potential) is sent. Once serotonin is in the synapse, three different actions can occur: 1) serotonin (5-HT) reuptake into the presynaptic neuron via the serotonin transporter; 2) 5-HT in the synapse is degraded into 5-HIAA via the enzyme monoamine oxidase (MAO); or 3) 5-HT binds to its postsynaptic neuron receptor (5-HT 1a, b, c, d, e; 2; 3; 4; 5; 6) where it ultimately leads to the regulation of gene expression and protein synthesis. Serotonin that is re-uptaken into the presynaptic neuron is either stored for subsequent release into the synapse again or is degraded by MAO into 5-HIAA. The action of the serotonin transporter is thus important because it determines the length of time 5-HT remains in the synapse. Antidepressants act on serotonin and norepinephrine transporters, to return neurotransmitter levels to those within the range of normal variation in a non-depressed population of individuals.



1.5 REFERENCES

1. Abrahamsson H. Gastrointestinal motility in patients with the irritable bowel syndrome. Scand J Gastroenterol Suppl 1987;130:21-6.

2. Mayer EA. Emerging disease model for functional gastrointestinal disorders. Am J Med 1999;107(5A):12S-19S.

3. Schmulson MW, Chang L. Diagnostic approach to the patient with irritable bowel syndrome. Am J Med 1999;107(5A):20S-26S.

4. Maxwell PR, Mendall MA, Kumar D. Irritable bowel syndrome. Lancet 1997;350(9092):1691-5.

5. Boyce PM, Koloski NA, Talley NJ. Irritable bowel syndrome according to varying diagnostic criteria: are the new Rome II criteria unnecessarily restrictive for research and practice? Am J Gastroenterol 2000;95(11):3176-83.

6. Kay L, Jorgensen T, Jensen KH. The epidemiology of irritable bowel syndrome in a random population: prevalence, incidence, natural history and risk factors. J Intern Med 1994;236(1):23-30.

7. Brandt LJ, Bjorkman D, Fennerty MB, Locke GR, Olden K, Peterson W, et al. Systematic review on the management of irritable bowel syndrome in North America. Am J Gastroenterol 2002;97(11 Suppl):S7-26.

8. Viera AJ, Hoag S, Shaughnessy J. Management of irritable bowel syndrome. Am Fam Physician 2002;66(10):1867-74.

9. Talley NJ, Gabriel SE, Harmsen WS, Zinsmeister AR, Evans RW. Medical costs in community subjects with irritable bowel syndrome. Gastroenterology 1995;109(6):1736-41.

10. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. Aliment Pharmacol Ther 2003;17(5):643-50.

11. Hammer J, Talley NJ. Diagnostic criteria for the irritable bowel syndrome. Am J Med 1999;107(5A):5S-11S.

12. Camilleri M. Therapeutic approach to the patient with irritable bowel syndrome. Am J Med 1999;107(5A):27S-32S.

13. Mertz H. Role of the brain and sensory pathways in gastrointestinal sensory disorders in humans. Gut 2002;51 Suppl 1:i29-33.

14. Camilleri M, Northcutt AR, Kong S, Dukes GE, McSorley D, Mangel AW. Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomised, placebo-controlled trial. Lancet 2000;355(9209):1035-40.

15. Scherl E, Frissora CL. Irritable bowel syndrome genophenomics: correlation of serotonin-transporter polymorphisms and alosetron response. Pharmacogenomics J 2003;3(2):64-6.

16. Mayer EA, Bradesi S. Alosetron and irritable bowel syndrome. Expert Opin Pharmacother 2003;4(11):2089-98.

17. Lembo A, Weber HC, Farraye FA. Alosetron in irritable bowel syndrome: strategies for its use in a common gastrointestinal disorder. Drugs 2003;63(18):1895-905.

18. Sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, et al. The burden of selected digestive diseases in the United States. Gastroenterology 2002;122(5):1500-11.

19. Whitehead WE SM, eds. Gastrointestinal Disorders: Behavioral and Physiological Basis for Treatment. New York, NY: Academic Press; 1985.

20. Hu WH, Wong WM, Lam CL, Lam KF, Hui WM, Lai KC, et al. Anxiety but not depression determines health care-seeking behaviour in Chinese patients with dyspepsia and irritable bowel syndrome: a population-based study. Aliment Pharmacol Ther 2002;16(12):2081-8.

21. Thompson WG, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. Dig Dis Sci 2002;47(1):225-35.

22. Leibbrand R, Cuntz U, Hiller W. Assessment of functional gastrointestinal disorders using the Gastro-Questionnaire. Int J Behav Med 2002;9(2):155-72.

23. Mearin F, Badia X, Balboa A, Baro E, Caldwell E, Cucala M, et al. Irritable bowel syndrome prevalence varies enormously depending on the employed diagnostic criteria: comparison of Rome II versus previous criteria in a general population. Scand J Gastroenterol 2001;36(11):1155-61.

24. Saito YA, Locke GR, Talley NJ, Zinsmeister AR, Fett SL, Melton LJ, 3rd. A comparison of the Rome and Manning criteria for case identification in epidemiological investigations of irritable bowel syndrome. Am J Gastroenterol 2000;95(10):2816-24.

25. Masud MA, Hasan M, Khan AK. Irritable bowel syndrome in a rural community in Bangladesh: prevalence, symptoms pattern, and health care seeking behavior. Am J Gastroenterol 2001;96(5):1547-52.

26. Chey WD, Olden K, Carter E, Boyle J, Drossman D, Chang L. Utility of the Rome I and Rome II criteria for irritable bowel syndrome in U.S. women. Am J Gastroenterol 2002;97(11):2803-11.

27. Camilleri M, Heading RC, Thompson WG. Clinical perspectives, mechanisms, diagnosis and management of irritable bowel syndrome. Aliment Pharmacol Ther 2002;16(8):1407-30.

28. Talley NJ, Boyce PM, Jones M. Predictors of health care seeking for irritable bowel syndrome: a population based study. Gut 1997;41(3):394-8.

29. Talley NJ, Howell S, Poulton R. The irritable bowel syndrome and psychiatric disorders in the community: is there a link? Am J Gastroenterol 2001;96(4):1072-9.

30. Kwan AC, Hu WH, Chan YK, Yeung YW, Lai TS, Yuen H. Prevalence of irritable bowel syndrome in Hong Kong. J Gastroenterol Hepatol 2002;17(11):1180-6.

31. Lau EM, Chan FK, Ziea ET, Chan CS, Wu JC, Sung JJ. Epidemiology of irritable bowel syndrome in Chinese. Dig Dis Sci 2002;47(11):2621-4.

32. Saito YA, Schoenfeld P, Locke GR, 3rd. The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol 2002;97(8):1910-5.

33. Locke GR, 3rd, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ. Risk factors for irritable bowel syndrome: role of analgesics and food sensitivities. Am J Gastroenterol 2000;95(1):157-65.

34. Simren M, Mansson A, Langkilde AM, Svedlund J, Abrahamsson H, Bengtsson U, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion 2001;63(2):108-15.

35. Lu CL, Chen CY, Chang FY, Lee SD. Characteristics of small bowel motility in patients with irritable bowel syndrome and normal humans: an Oriental study. Clin Sci (Lond) 1998;95(2):165-9.

36. Baretic M, Bilic A, Jurcic D, Mihanovic M, Sunic-Omejc M, Dorosulic Z, et al. Epidemiology of irritable bowel syndrome in Croatia. Coll Antropol 2002;26 Suppl:85-91.

37. Blanchard EB, Scharff L, Schwarz SP, Suls JM, Barlow DH. The role of anxiety and depression in the irritable bowel syndrome. Behav Res Ther 1990;28(5):401-5.

38. Swiatkowski M, Rybakowski JK. Depression and T lymphocytes in patients with irritable bowel syndrome. J Affect Disord 1993;28(3):199-202.

39. Masand PS, Kaplan DS, Gupta S, Bhandary AN, Nasra GS, Kline MD, et al. Major depression and irritable bowel syndrome: is there a relationship? J Clin Psychiatry 1995;56(8):363-7.

40. Hui WM, Shiu LP, Lam SK. The perception of life events and daily stress in nonulcer dyspepsia. Am J Gastroenterol 1991;86(3):292-6.

41. Harris A, Martin BJ. Increased abdominal pain during final examinations. Dig Dis Sci 1994;39(1):104-8.

42. Langeluddecke P, Goulston K, Tennant C. Psychological factors in dyspepsia of unknown cause: a comparison with peptic ulcer disease. J Psychosom Res 1990;34(2):215-22.

43. Talley NJ, Fung LH, Gilligan IJ, McNeil D, Piper DW. Association of anxiety, neuroticism, and depression with dyspepsia of unknown cause. A case-control study. Gastroenterology 1986;90(4):886-92.

44. Talley NJ, Jones M, Piper DW. Psychosocial and childhood factors in essential dyspepsia. A case-control study. Scand J Gastroenterol 1988;23(3):341-6.

45. Veale D, Kavanagh G, Fielding JF, Fitzgerald O. Primary fibromyalgia and the irritable bowel syndrome: different expressions of a common pathogenetic process. Br J Rheumatol 1991;30(3):220-2.

46. Pace F, Sarzi-Puttini P, Manzionna G, Molteni P, Turiel M, Panni B, et al. Visceral hypersensitivity is not a feature of fibromyalgia syndrome. Journal of Musculoskeletal Pain 2001;9(1):47-55.

47. Sperber AD, Atzmon Y, Neumann L, Weisberg I, Shalit Y, Abu-Shakrah M, et al. Fibromyalgia in the irritable bowel syndrome: studies of prevalence and clinical implications. Am J Gastroenterol 1999;94(12):3541-6.

48. Aaron LA, Buchwald D. A review of the evidence for overlap among unexplained clinical conditions. Ann Intern Med 2001;134(9 Pt 2):868-81.

49. Aaron LA, Buchwald D. Chronic diffuse musculoskeletal pain, fibromyalgia and co-morbid unexplained clinical conditions. Best Pract Res Clin Rheumatol 2003;17(4):563-74.

50. Aaron LA, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. Arch Intern Med 2000;160(2):221-7.

51. Clauw DJ. The pathogenesis of chronic pain and fatigue syndromes, with special reference to fibromyalgia. Med Hypotheses 1995;44(5):369-78.

52. Azpiroz F, Dapoigny M, Pace F, Muller-Lissner S, Coremans G, Whorwell P, et al. Nongastrointestinal disorders in the irritable bowel syndrome. Digestion 2000;62(1):66-72.

53. Hudson JI, Goldenberg DL, Pope HG, Jr., Keck PE, Jr., Schlesinger L. Comorbidity of fibromyalgia with medical and psychiatric disorders. Am J Med 1992;92(4):363-7.

54. Aaron LA, Herrell R, Ashton S, Belcourt M, Schmaling K, Goldberg J, et al. Comorbid clinical conditions in chronic fatigue: a co-twin control study. J Gen Intern Med 2001;16(1):24-31.

55. Gomborone JE, Gorard DA, Dewsnap PA, Libby GW, Farthing MJ. Prevalence of irritable bowel syndrome in chronic fatigue. J R Coll Physicians Lond 1996;30(6):512-3.

56. Roussos A, Koursarakos P, Patsopoulos D, Gerogianni I, Philippou N. Increased prevalence of irritable bowel syndrome in patients with bronchial asthma. Respir Med 2003;97(1):75-9.

57. Yazar A, Atis S, Konca K, Pata C, Akbay E, Calikoglu M, et al. Respiratory symptoms and pulmonary functional changes in patients with irritable bowel syndrome. Am J Gastroenterol 2001;96(5):1511-6.

58. Huerta C, Garcia Rodriguez LA, Wallander MA, Johansson S. Risk of irritable bowel syndrome among asthma patients. Pharmacoepidemiol Drug Saf 2002;11(1):31-5.

59. Kennedy TM, Jones RH, Hungin AP, O'Flanagan H, Kelly P. Irritable bowel syndrome, gastro-oesophageal reflux, and bronchial hyper-responsiveness in the general population. Gut 1998;43(6):770-4.

60. Locke GR, 3rd, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ, 3rd. Familial association in adults with functional gastrointestinal disorders. Mayo Clin Proc 2000;75(9):907-12.

61. 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(4):799-804.

62. Hahn BA, Kirchdoerfer LJ, Fullerton S, Mayer E. Patient-perceived severity of irritable bowel syndrome in relation to symptoms, health resource utilization and quality of life. Aliment Pharmacol Ther 1997;11(3):553-9.

63. Hahn BA, Yan S, Strassels S. Impact of irritable bowel syndrome on quality of life and resource use in the United States and United Kingdom. Digestion 1999;60(1):77-81.

64. Luscombe FA. Health-related quality of life and associated psychosocial factors in irritable bowel syndrome: a review. Qual Life Res 2000;9(2):161-76.

65. Frank L, Kleinman L, Rentz A, Ciesla G, Kim JJ, Zacker C. Health-related quality of life associated with irritable bowel syndrome: comparison with other chronic diseases. Clin Ther 2002;24(4):675-89; discussion 674.

66. Li FX, Patten SB, Hilsden RJ, Sutherland LR. Irritable bowel syndrome and health-related quality of life: a population-based study in Calgary, Alberta. Can J Gastroenterol 2003;17(4):259-63.

67. El-Serag HB, Olden K, Bjorkman D. Health-related quality of life among persons with irritable bowel syndrome: a systematic review. Aliment Pharmacol Ther 2002;16(6):1171-85.

68. Halder SL, Locke GR, 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ, 3rd. Impact of functional gastrointestinal disorders on health-related quality of life: a population-based case-control study. Aliment Pharmacol Ther 2004;19(2):233-42.

69. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. Jama 2004;292(7):852-8.

70. Burr RL, Heitkemper M, Jarrett M, Cain KC. Comparison of autonomic nervous system indices based on abdominal pain reports in women with irritable bowel syndrome. Biol Res Nurs 2000;2(2):97-106.

71. Houghton LA, Foster JM, Whorwell PJ. Alosetron, a 5-HT3 receptor antagonist, delays colonic transit in patients with irritable bowel syndrome and healthy volunteers. Aliment Pharmacol Ther 2000;14(6):775-82.

72. Ritchie J. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. Gut 1973;14(2):125-32.

73. Silverman DH, Munakata JA, Ennes H, Mandelkern MA, Hoh CK, Mayer EA. Regional cerebral activity in normal and pathological perception of visceral pain. Gastroenterology 1997;112(1):64-72.

74. Aggarwal A, Cutts TF, Abell TL, Cardoso S, Familoni B, Bremer J, et al. Predominant symptoms in irritable bowel syndrome correlate with specific autonomic nervous system abnormalities. Gastroenterology 1994;106(4):945-50.

75. Kim DY, Camilleri M. Serotonin: a mediator of the brain-gut connection. Am J Gastroenterol 2000;95(10):2698-709.

76. Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, et al. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002;123(2):425-32.

77. von der Ohe MR, Hanson RB, Camilleri M. Serotonergic mediation of postprandial colonic tonic and phasic responses in humans. Gut 1994;35(4):536-41.

78. Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS). Patients' description of diarrhea, constipation and symptom variation during a prospective 6-week study. Eur J Gastroenterol Hepatol 1998;10(5):415-21.

79. Choi MG, Camilleri M, O'Brien MD, Kammer PP, Hanson RB. A pilot study of motility and tone of the left colon in patients with diarrhea due to functional disorders and dysautonomia. Am J Gastroenterol 1997;92(2):297-302.

80. Borman RA, Tilford NS, Harmer DW, Day N, Ellis ES, Sheldrick RL, et al. 5-HT(2B) receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro. Br J Pharmacol 2002;135(5):1144-51.

81. Pata C, Erdal ME, Derici E, Yazar A, Kanik A, Ulu O. Serotonin transporter gene polymorphism in irritable bowel syndrome. Am J Gastroenterol 2002;97(7):1780-4.

82. Kalantar JS, Locke GR, 3rd, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. Gut 2003;52(12):1703-7.

83. Kalantar JS, Locke GR, 3rd, Talley NJ, Zinsmeister AR, Fett SL, Melton LJ, 3rd. Is irritable bowel syndrome more likely to be persistent in those with relatives who suffer from gastrointestinal symptoms? A population-based study at three time points. Aliment Pharmacol Ther 2003;17(11):1389-97.

84. Whorwell PJ, McCallum M, Creed FH, Roberts CT. Non-colonic features of irritable bowel syndrome. Gut 1986;27(1):37-40.

85. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet 2002;3(11):872-82.

86. Plomin R, DeFries J, McClearn G, McGuffin P. Behavioral Genetics. Fourth Edition ed. New York: Worth Publishers; 2001.

87. Martin N, Boomsma D, Machin G. A twin-pronged attack on complex traits. Nat Genet 1997;17(4):387-92.

88. Neale MC, Maes HH. Methodology for genetic studies of twins and families. Dordrecht, The Netherlands: Kluwer Academic Publishers; in progress.

89. Neale MC, Boker SM, Xie G, Maes HH. Mx: Statistical Modeling. 5th edition ed. Richmond, VA: Department of Psychiatry, Medical College of Virginia, Virginia Commonwealth University; 1999.

90. Svedberg P, Johansson S, Wallander MA, Hamelin B, Pedersen NL. Extra-intestinal manifestations associated with irritable bowel syndrome: a twin study. Aliment Pharmacol Ther 2002;16(5):975-83.

91. 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(8):1311-7.

92. Mohammed I, Cherkas LF, Riley SA, Spector TD, Trudgill NJ. Genetic influences in irritable bowel syndrome: a twin study. Am J Gastroenterol 2005;100(6):1340-4.

93. Khoury MJ, Beaty TH, Cohen BH. Fundamentals of Genetic Epidemiology. New York, NY: Oxford University Press; 1993.

94. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361(9357):598-604.

95. Fava M, Kendler KS. Major depressive disorder. Neuron 2000;28(2):335-41.

96. Bebbington P, Dunn G, Jenkins R, Lewis G, Brugha T, Farrell M, et al. The influence of age and sex on the prevalence of depressive conditions: report from the National Survey of Psychiatric Morbidity. Int Rev Psychiatry 2003;15:74-83.

97. Patten SB. Long-term medical conditions and major depression in a Canadian population study at waves 1 and 2. J Affect Disord 2001;63(1-3):35-41.

98. Roberts RE, Lewinsohn PM, Seeley JR. Symptoms of DSM-III-R major depression in adolescence: evidence from an epidemiological survey. J Am Acad Child Adolesc Psychiatry 1995;34(12):1608-17.

99. Angst J, Baastrup P, Grof P, Hippius H, Poldinger W, Weis P. The course of monopolar depression and bipolar psychoses. Psychiatr Neurol Neurochir 1973;76(6):489-500.

100. Mueller TI, Leon AC. Recovery, chronicity, and levels of psychopathology in major depression. Psychiatr Clin North Am 1996;19(1):85-102.

101. Mueller TI, Leon AC, Keller MB, Solomon DA, Endicott J, Coryell W, et al. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am J Psychiatry 1999;156(7):1000-6.

102. Solomon DA, Keller MB, Leon AC, Mueller TI, Lavori PW, Shea MT, et al. Multiple recurrences of major depressive disorder. Am J Psychiatry 2000;157(2):229-33.

103. Burt VK, Stein K. Epidemiology of depression throughout the female life cycle. J Clin Psychiatry 2002;63 Suppl 7:9-15.

104. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51(1):8-19.

105. Pincus HA, Pettit AR. The societal costs of chronic major depression. J Clin Psychiatry 2001;62 Suppl 6:5-9.

106. Katon WJ. Clinical and health services relationships between major depression, depressive symptoms, and general medical illness. Biol Psychiatry 2003;54(3):216-26.

107. Weissman MM. Advances in psychiatric epidemiology: rates and risks for major depression. Am J Public Health 1987;77(4):445-51.

108. Osby U, Brandt L, Correia N, Ekbom A, Sparen P. Excess mortality in bipolar and unipolar disorder in Sweden. Arch Gen Psychiatry 2001;58(9):844-50.

109. Cuijpers P, Smit F. Excess mortality in depression: a meta-analysis of community studies. J Affect Disord 2002;72(3):227-36.

110. Williams JB, Gibbon M, First MB, Spitzer RL, Davies M, Borus J, et al. The Structured Clinical Interview for DSM-III-R (SCID). II. Multisite test-retest reliability. Arch Gen Psychiatry 1992;49(8):630-6.

111. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). Jama 2003;289(23):3095-105.

112. Angst J. The epidemiology of depressive disorders. Eur Neuropsychopharmacol 1995;5 Suppl:95-8.

113. Tondo L, Burrai C, Scamonatti L, Weissenburger J, Rush J. Comparison between clinician-rated and self-reported depressive symptoms in Italian psychiatric patients. Neuropsychobiology 1988;19(1):1-5.

114. Spitzer RL, Endicott J, Robins E. Research diagnostic criteria: rationale and reliability. Arch Gen Psychiatry 1978;35(6):773-82.

115. Lerer B, Macciardi F, Segman RH, Adolfsson R, Blackwood D, Blairy S, et al. Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 2001;6(5):579-85.

116. Schulze TG, Muller DJ, Krauss H, Scherk H, Ohlraun S, Syagailo YV, et al. Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder. Am J Med Genet 2000;96(6):801-3.

117. Neiswanger K, Zubenko GS, Giles DE, Frank E, Kupfer DJ, Kaplan BB. Linkage and association analysis of chromosomal regions containing genes related to neuroendocrine or serotonin function in families with early-onset, recurrent major depression. Am J Med Genet 1998;81(5):443-9.

118. Zimmerman M, Coryell W, Corenthal C, Wilson S. A self-report scale to diagnose major depressive disorder. Arch Gen Psychiatry 1986;43(11):1076-81.

119. Hodgins DC, Dufour M, Armstrong S. The reliability and validity of the inventory to diagnose depression in alcohol-dependent men and women. J Subst Abuse 2000;11(4):369-78.

120. Weissman MM, Leaf PJ, Holzer CE, 3rd, Myers JK, Tischler GL. The epidemiology of depression. An update on sex differences in rates. J Affect Disord 1984;7(3-4):179-88.

121. Terluin B, van Hout HP, van Marwijk HW, Ader HJ, van der Meer K, de Haan M, et al. Reliability and validity of the assessment of depression in general practice: the Short Depression Interview (SDI). Gen Hosp Psychiatry 2002;24(6):396-405.

122. Sheeran T, Zimmerman M. Case identification of depression with self-report questionnaires. Psychiatry Res 2002;109(1):51-9.

123. Bland RC. Epidemiology of affective disorders: a review. Can J Psychiatry 1997;42(4):367-77.

124. Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, et al. Cross-national epidemiology of major depression and bipolar disorder. Jama 1996;276(4):293-9.

125. Patten SB, Stuart HL, Russell ML, Maxwell CJ, Arboleda-Florez J. Epidemiology of major depression in a predominantly rural health region. Soc Psychiatry Psychiatr Epidemiol 2003;38(7):360-5.

126. Wilhelm K, Mitchell P, Slade T, Brownhill S, Andrews G. Prevalence and correlates of DSM-IV major depression in an Australian national survey. J Affect Disord 2003;75(2):155-62.

127. Kessler RC. Epidemiology of women and depression. J Affect Disord 2003;74(1):5-13.

128. Regier DA, Narrow WE, Rae DS, Manderscheid RW, Locke BZ, Goodwin FK. The de facto US mental and addictive disorders service system. Epidemiologic catchment area prospective 1-year prevalence rates of disorders and services. Arch Gen Psychiatry 1993;50(2):85-94.

129. Blazer DG, Kessler RC, McGonagle KA, Swartz MS. The prevalence and distribution of major depression in a national community sample: the National Comorbidity Survey. Am J Psychiatry 1994;151(7):979-86.

130. Kendler KS, Gardner CO, Prescott CA. Toward a comprehensive developmental model for major depression in women. Am J Psychiatry 2002;159:1133-1145.

131. Hudson JI, Mangweth B, Pope HG, Jr., De Col C, Hausmann A, Gutweniger S, et al. Family study of affective spectrum disorder. Arch Gen Psychiatry 2003;60(2):170-7.

132. Stoline AM. To sleep--perchance to dream ... of affective disorders. Am J Psychiatry 1996;153(1):134-5.

133. Meltzer H. Serotonergic dysfunction in depression. Br J Psychiatry Suppl 1989(8):25-31.

134. Zill P, Engel R, Baghai TC, Juckel G, Frodl T, Muller-Siecheneder F, et al. Identification of a naturally occurring polymorphism in the promoter region of the norepinephrine transporter and analysis in major depression. Neuropsychopharmacology 2002;26(4):489-93.

135. Mann JJ. Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. Neuropsychopharmacology 1999;21(2 Suppl):99S-105S.

136. Tadic A, Rujescu D, Szegedi A, Giegling I, Singer P, Moller HJ, et al. Association of a MAOA gene variant with generalized anxiety disorder, but not with panic disorder or major depression. Am J Med Genet 2003;117B(1):1-6.

137. Dursun SM, Blackburn JR, Kutcher SP. An exploratory approach to the serotonergic hypothesis of depression: bridging the synaptic gap. Med Hypotheses 2001;56(2):235-43.

138. Kramlinger K. Mayo Clinic on Depression. New York, NY: Kensington Publishing Corporation; 2001.

139. American Medical Association Essential Guide to Depression. New York, NY: Pocket Books; 1998.

140. Leonard BE. Fundamentals of Psychopharmacology. 2nd Edition ed. New York, NY: John Wiley & Sons; 1997.

141. Staley JK, Malison RT, Innis RB. Imaging of the serotonergic system: interactions of neuroanatomical and functional abnormalities of depression. Biol Psychiatry 1998;44(7):534-49.

142. Owen D, Du L, Bakish D, Lapierre YD, Hrdina PD. Norepinephrine transporter gene polymorphism is not associated with susceptibility to major depression. Psychiatry Res 1999;87(1):1-5.

143. Charney DS. Monoamine dysfunction and the pathophysiology and treatment of depression. J Clin Psychiatry 1998;59 Suppl 14:11-4.

144. Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, et al. Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci 1997;17(21):8451-8.

145. De Bellis MD, Geracioti TD, Jr., Altemus M, Kling MA. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. Biol Psychiatry 1993;33(8-9):636-41.

146. Lee CM, Javitch JA, Snyder SH. Recognition sites for norepinephrine uptake: regulation by neurotransmitter. Science 1983;220(4597):626-9.

147. Lake CR, Pickar D, Ziegler MG, Lipper S, Slater S, Murphy DL. High plasma norepinephrine levels in patients with major affective disorder. Am J Psychiatry 1982;139(10):1315-8.

148. Roy A, Pickar D, Linnoila M, Potter WZ. Plasma norepinephrine level in affective disorders. Relationship to melancholia. Arch Gen Psychiatry 1985;42(12):1181-5.

149. Veith RC, Lewis N, Linares OA, Barnes RF, Raskind MA, Villacres EC, et al. Sympathetic nervous system activity in major depression. Basal and desipramine-induced alterations in plasma norepinephrine kinetics. Arch Gen Psychiatry 1994;51(5):411-22.

150. Hong CJ, Tsai SJ, Cheng CY, Liao WY, Song HL, Lai HC. Association analysis of the 5-HT(6) receptor polymorphism (C267T) in mood disorders. Am J Med Genet 1999;88(6):601-2.

151. Baldwin D, Rudge S. The role of serotonin in depression and anxiety. Int Clin Psychopharmacol 1995;9 Suppl 4:41-5.

152. Struder HK, Weicker H. Physiology and pathophysiology of the serotonergic system and its implications on mental and physical performance. Part I. Int J Sports Med 2001;22(7):467-81.

153. Yatham LN, Liddle PF, Shiah IS, Scarrow G, Lam RW, Adam MJ, et al. Brain serotonin2 receptors in major depression: a positron emission tomography study. Arch Gen Psychiatry 2000;57(9):850-8.

154. The Molecular and Genetic Basis of Neurologic and Psychiatric Disease. 3rd ed. Philadelphia, PA: Butterworth Heinemann; 2003.

155. Asberg M, Thoren P, Traskman L, Bertilsson L, Ringberger V. "Serotonin depression"--a biochemical subgroup within the affective disorders? Science 1976;191(4226):478-80.

156. Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 1994;40(2):288-95.

157. Arango V, Underwood MD, Mann JJ. Serotonin brain circuits involved in major depression and suicide. Prog Brain Res 2002;136:443-53.

158. Linner L, Wiker C, Arborelius L, Schalling M, Svensson TH. Selective noradrenaline reuptake inhibition enhances serotonergic neuronal activity and transmitter release in the rat forebrain. J Neural Transm 2004;111(2):127-39.

159. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157(10):1552-62.

160. Weissman MM, Gammon GD, John K, Merikangas KR, Warner V, Prusoff BA, et al. Children of depressed parents. Increased psychopathology and early onset of major depression. Arch Gen Psychiatry 1987;44(10):847-53.

161. Hammen C, Brennan PA. Severity, chronicity, and timing of maternal depression and risk for adolescent offspring diagnoses in a community sample. Arch Gen Psychiatry 2003;60(3):253-8.

162. Lieb R, Isensee B, Hofler M, Pfister H, Wittchen HU. Parental major depression and the risk of depression and other mental disorders in offspring: a prospective-longitudinal community study. Arch Gen Psychiatry 2002;59(4):365-74.

163. Klein DN, Lewinsohn PM, Seeley JR, Rohde P. A family study of major depressive disorder in a community sample of adolescents. Arch Gen Psychiatry 2001;58(1):13-20.

164. Klein DN, Shankman SA, Lewinsohn PM, Rohde P, Seeley JR. Family study of chronic depression in a community sample of young adults. Am J Psychiatry 2004;161(4):646-53.

165. von Knorring AL, Cloninger CR, Bohman M, Sigvardsson S. An adoption study of depressive disorders and substance abuse. Arch Gen Psychiatry 1983;40(9):943-50.

166. Cadoret RJ, O'Gorman TW, Heywood E, Troughton E. Genetic and environmental factors in major depression. J Affect Disord 1985;9(2):155-64.

167. Wender PH, Kety SS, Rosenthal D, Schulsinger F, Ortmann J, Lunde I. Psychiatric disorders in the biological and adoptive families of adopted individuals with affective disorders. Arch Gen Psychiatry 1986;43(10):923-9.

168. Fu Q, Heath AC, Bucholz KK, Nelson E, Goldberg J, Lyons MJ, et al. Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. Arch Gen Psychiatry 2002;59(12):1125-32.

169. Kendler KS, Aggen SH. Time, memory and the heritability of major depression. Psychol Med 2001;31(5):923-8.

170. Zubenko GS, Hughes IH, Stiffler JS, Zubenko WN, Kaplan BB. D2S2944 identifies a likely susceptibility locus for recurrent, early-onset, major depression in women. Mol Psychiatry 2002;7(5):460-7.

171. Zubenko GS, Hughes HB, 3rd, Maher BS, Stiffler JS, Zubenko WN, Marazita ML. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset, major depression. Am J Med Genet 2002;114(8):980-7.

172. Abkevich V, Camp NJ, Hensel CH, Neff CD, Russell DL, Hughes DC, et al. Predisposition locus for major depression at chromosome 12q22-12q23.2. Am J Hum Genet 2003;73(6):1271-81.

173. Zubenko GS, Maher B, Hughes HB, 3rd, Zubenko WN, Stiffler JS, Kaplan BB, et al. Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. Am J Med Genet 2003;123B(1):1-18.

174. Holmans P, Zubenko GS, Crowe RR, DePaulo Jr. JR, Scheftner WA, Weissman MM, et al. Genomewide significant linkage to recurrent, early-onset major depressive disorder on chromosome 15q. Am J Hum Genet 2004;74:1154-1167.

175. Collier DA, Arranz MJ, Sham P, Battersby S, Vallada H, Gill P, et al. The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. Neuroreport 1996;7(10):1675-9.

176. Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch Gen Psychiatry 2000;57(8):729-38.

177. Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P, et al. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol Psychiatry 1997;2(5):398-402.

178. Hauser J, Leszczynska A, Samochowiec J, Czerski PM, Ostapowicz A, Chlopocka M, et al. Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. Eur Psychiatry 2003;18(3):129-32.

179. Ogilvie AD, Battersby S, Bubb VJ, Fink G, Harmar AJ, Goodwim GM, et al. Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 1996;347(9003):731-3.

180. Liu W, Gu N, Feng G, Li S, Bai S, Zhang J, et al. Tentative association of the serotonin transporter with schizophrenia and unipolar depression but not with bipolar disorder in Han Chinese. Pharmacogenetics 1999;9(4):491-5.

181. Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES, et al. Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. Am J Med Genet 1998;81(1):58-63.

182. Papadimitriou GN, Dikeos DG, Souery D, Del-Favero J, Massat I, Avramopoulos D, et al. Genetic association between the phospholipase A2 gene and unipolar affective disorder: a multicentre case-control study. Psychiatr Genet 2003;13(4):211-20.

183. Tan EC, Chan AO, Tan CH, Mahendran R, Wang A, Chua HC. Case-control and linkage disequilibrium studies of the tryptophan hydroxylase gene polymorphisms and major depressive disorder. Psychiatr Genet 2003;13(3):151-4.

184. Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, et al. Dopamine D2, D3 and D4 receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 1996;40(1-2):7-13.

185. Huang YY, Oquendo MA, Friedman JM, Greenhill LL, Brodsky B, Malone KM, et al. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology 2003;28(1):163-9.

186. Llerena A, Berecz R, Dorado P, Gonzalez AP, Penas LEM, De La Rubia A. CYP2C9 gene and susceptibility to major depressive disorder. Pharmacogenomics J 2003;3(5):300-2.

187. Qian Y, Lin S, Jiang S, Jiang K, Wu X, Tang G, et al. Studies of the DXS7 polymorphism at the MAO loci in unipolar depression. Am J Med Genet 1999;88(6):598-600.

188. Villafuerte SM, Del-Favero J, Adolfsson R, Souery D, Massat I, Mendlewicz J, et al. Gene-based SNP genetic association study of the corticotropin-releasing hormone receptor-2 (CRHR2) in major depression. Am J Med Genet 2002;114(2):222-6.

189. Zill P, Baghai TC, Zwanzger P, Schule C, Eser D, Rupprecht R, et al. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Mol Psychiatry 2004.

190. Henkel V, Baghai TC, Eser D, Zill P, Mergl R, Zwanzger P, et al. The gamma amino butyric acid (GABA) receptor alpha-3 subunit gene polymorphism in unipolar depressive disorder: a genetic association study. Am J Med Genet 2004;126B(1):82-7.

191. Furlong RA, Rubinsztein JS, Ho L, Walsh C, Coleman TA, Muir WJ, et al. Analysis and metaanalysis of two polymorphisms within the tyrosine hydroxylase gene in bipolar and unipolar affective disorders. Am J Med Genet 1999;88(1):88-94.

192. Souery D, Lipp O, Mahieu B, Mendelbaum K, De Bruyn A, De Maertelaer V, et al. Excess tyrosine hydroxylase restriction fragment length polymorphism homozygosity in unipolar but not bipolar patients: a preliminary report. Biol Psychiatry 1996;40(4):305-8.

193. Arias B, Gutierrez B, Pintor L, Gasto C, Fananas L. Variability in the 5-HT(2A) receptor gene is associated with seasonal pattern in major depression. Mol Psychiatry 2001;6(2):239-42.

194. Zill P, Engel R, Baghai TC, Zwanzger P, Schule C, Minov C, et al. Analysis of polymorphisms in the olfactory G-protein Golf in major depression. Psychiatr Genet 2002;12(1):17-22.

195. Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry 2003;8(6):574-91.

196. Rothman KJ, Greenland S. Modern Epidemiology. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998.

197. Stober G, Heils A, Lesch KP. Serotonin transporter gene polymorphism and affective disorder. Lancet 1996;347(9011):1340-1.

198. Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T, et al. Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Mol Psychiatry 1997;2(6):457-62.

199. Kunugi H, Tatsumi M, Sakai T, Hattori M, Nanko S. Serotonin transporter gene polymorphism and affective disorder. Lancet 1996;347(9011):1340.

200. Hoehe MR, Wendel B, Grunewald I, Chiaroni P, Levy N, Morris-Rosendahl D, et al. Serotonin transporter (5-HTT) gene polymorphisms are not associated with susceptibility to mood disorders. Am J Med Genet 1998;81(1):1-3.

201. Gutierrez B, Pintor L, Gasto C, Rosa A, Bertranpetit J, Vieta E, et al. Variability in the serotonin transporter gene and increased risk for major depression with melancholia. Hum Genet 1998;103(3):319-22.

202. Mellerup E, Bennike B, Bolwig T, Dam H, Hasholt L, Jorgensen MB, et al. Platelet serotonin transporters and the transporter gene in control subjects, unipolar patients and bipolar patients. Acta Psychiatr Scand 2001;103(3):229-33.

203. Towers AL, Burgio KL, Locher JL, Merkel IS, Safaeian M, Wald A. Constipation in the elderly: influence of dietary, psychological, and physiological factors. J Am Geriatr Soc 1994;42(7):701-6.

204. Mayer EA, Craske M, Naliboff BD. Depression, anxiety, and the gastrointestinal system. J Clin Psychiatry 2001;62 Suppl 8:28-36; discussion 37.

205. Lydiard RB. Irritable bowel syndrome, anxiety, and depression: what are the links? J Clin Psychiatry 2001;62 Suppl 8:38-45; discussion 46-7.

206. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? Gastroenterology 2002;122(4):1140-56.

207. Walker EA, Katon WJ, Jemelka RP, Roy-Bryne PP. Comorbidity of gastrointestinal complaints, depression, and anxiety in the Epidemiologic Catchment Area (ECA) Study. Am J Med 1992;92(1A):26S-30S.

208. Camilleri M, Choi MG. Review article: irritable bowel syndrome. Aliment Pharmacol Ther 1997;11(1):3-15.

209. Sullivan G, Jenkins PL, Blewett AE. Irritable bowel syndrome and family history of psychiatric disorder: a preliminary study. Gen Hosp Psychiatry 1995;17(1):43-6.

210. Hudson JI, Pope HG, Jr. Affective spectrum disorder: does antidepressant response identify a family of disorders with a common pathophysiology? Am J Psychiatry 1990;147(5):552-64.

211. Woodman CL, Breen K, Noyes R, Jr., Moss C, Fagerholm R, Yagla SJ, et al. The relationship between irritable bowel syndrome and psychiatric illness. A family study. Psychosomatics 1998;39(1):45-54.

212. Gruber AJ, Hudson JI, Pope HG, Jr. The management of treatment-resistant depression in disorders on the interface of psychiatry and medicine. Fibromyalgia, chronic fatigue syndrome, migraine, irritable bowel syndrome, atypical facial pain, and premenstrual dysphoric disorder. Psychiatr Clin North Am 1996;19(2):351-69.

213. Lesch KP, Mossner R. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol Psychiatry 1998;44(3):179-92.

214. Oster J. Recurrent abdominal pain, headache and limb pains in children and adolescents. Pediatrics 1972;50(3):429-36.

215. Schulte CM, Goebell H, Roher HD, Schulte KM. C-509T polymorphism in the TGFB1 gene promoter: impact on Crohn's disease susceptibility and clinical course? Immunogenetics 2001;53(2):178-82.

216. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut 2003;52(1):91-3.

217. Kessler RC, Walters EE. Epidemiology of DSM-III-R major depression and minor depression among adolescents and young adults in the National Comorbidity Survey. Depress Anxiety 1998;7(1):3-14.

218. Tsuang MT, Winokur G, Crowe RR. Morbidity risks of schizophrenia and affective disorders among first degree relatives of patients with schizophrenia, mania, depression and surgical conditions. Br J Psychiatry 1980;137:497-504.

219. Gershon ES, Hamovit J, Guroff JJ, Dibble E, Leckman JF, Sceery W, et al. A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. Arch Gen Psychiatry 1982;39(10):1157-67.

220. Weissman MM, Gershon ES, Kidd KK, Prusoff BA, Leckman JF, Dibble E, et al. Psychiatric disorders in the relatives of probands with affective disorders. The Yale University--National Institute of Mental Health Collaborative Study. Arch Gen Psychiatry 1984;41(1):13-21.

221. Maier W, Lichtermann D, Minges J, Hallmayer J, Heun R, Benkert O, et al. Continuity and discontinuity of affective disorders and schizophrenia. Results of a controlled family study. Arch Gen Psychiatry 1993;50(11):871-83.

222. Weissman MM, Wickramaratne P, Adams PB, Lish JD, Horwath E, Charney D, et al. The relationship between panic disorder and major depression. A new family study. Arch Gen Psychiatry 1993;50(10):767-80.

223. McGuffin P, Katz R, Watkins S, Rutherford J. A hospital-based twin register of the heritability of DSM-IV unipolar depression. Arch Gen Psychiatry 1996;53(2):129-36.

224. Kendler KS, Pedersen NL, Neale MC, Mathe AA. A pilot Swedish twin study of affective illness including hospital- and population-ascertained subsamples: results of model fitting. Behav Genet 1995;25(3):217-32.

225. Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, et al. A registry-based twin study of depression in men. Arch Gen Psychiatry 1998;55(5):468-72.

226. Bierut LJ, Heath AC, Bucholz KK, Dinwiddie SH, Madden PA, Statham DJ, et al. Major depressive disorder in a community-based twin sample: are there different genetic and environmental contributions for men and women? Arch Gen Psychiatry 1999;56(6):557-63.

227. Kendler KS, Prescott CA. A population-based twin study of lifetime major depression in men and women. Arch Gen Psychiatry 1999;56(1):39-44.

228. Kendler KS, Gardner CO, Prescott CA. Corrections to 2 prior published articles. Arch Gen Psychiatry 2000;57(1):94-5.

229. Balciuniene J, Yuan QP, Engstrom C, Lindblad K, Nylander PO, Sundvall M, et al. Linkage analysis of candidate loci in families with recurrent major depression. Mol Psychiatry 1998;3(2):162-8.

230. Wang Z, Crowe RR, Tanna VL, Winokur G. Alpha2 adrenergic receptor subtypes in depression: A candidate gene study. J Affect Disord 1992;25:191-196.

231. Seretti A, Cusin C, Lattuada E, Di Bella D, Catalano M, Smeraldi E. Serotonin transporter gene (5-HTTLPR) is not associated with depressive symptomatology in mood disorders. Mol Psychiatry 1999;4(3):280-3.

232. Minov C, Baghai TC, Schule C, Zwanzger P, Schwarz MJ, Zill P, et al. Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression. Neurosci Lett 2001;303(2):119-22.

233. Oswald P, Souery D, Massat I, Del-Favero J, Linotte S, Papadimitriou G, et al. Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. Eur Neuropsychopharmacol 2003;13(5):365-8.

234. Arias B, Collier DA, Gasto C, Pintor L, Gutierrez B, Valles V, et al. Genetic variation in the 5-HT5A receptor gene in patients with bipolar disorder and major depression. Neurosci Lett 2001;303(2):111-4.

235. Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, et al. Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry 1999;4(4):389-92.

236. Furlong RA, Coleman TA, Ho L, Rubinsztein JS, Walsh C, Paykel ES, et al. No association of a functional polymorphism in the dopamine D2 receptor promoter region with bipolar or unipolar affective disorders. Am J Med Genet 1998;81(5):385-7.

237. Massat I, Souery D, Del-Favero J, Van Gestel S, Serretti A, Macciardi F, et al. Positive association of dopamine D2 receptor polymorphism with bipolar affective disorder in a European Multicenter Association Study of affective disorders. Am J Med Genet 2002;114(2):177-85.

238. Serretti A, Lilli R, Di Bella D, Bertelli S, Nobile M, Novelli E, et al. Dopamine receptor D4 gene is not associated with major psychoses. Am J Med Genet 1999;88(5):486-91.

239. Oruc L, Verheyen GR, Furac I, Jakovljevic M, Ivezic S, Raeymaekers P, et al. Analysis of the tyrosine hydroxylase and dopamine D4 receptor genes in a Croatian sample of bipolar I and unipolar patients. Am J Med Genet 1997;74(2):176-8.

240. Syagailo YV, Stober G, Grassle M, Reimer E, Knapp M, Jungkunz G, et al. Association analysis of the functional monoamine oxidase A gene promoter polymorphism in psychiatric disorders. Am J Med Genet 2001;105(2):168-71.

241. Furlong RA, Ho L, Rubinsztein JS, Walsh C, Paykel ES, Rubinsztein DC. No association of the tryptophan hydroxylase gene with bipolar affective disorder, unipolar affective disorder, or suicidal behaviour in major affective disorder. Am J Med Genet 1998;81(3):245-7.

242. Desan PH, Oren DA, Malison R, Price LH, Rosenbaum J, Smoller J, et al. Genetic polymorphism at the CLOCK gene locus and major depression. Am J Med Genet 2000;96(3):418-21.

243. Ryu SH, Lee SH, Lee HJ, Cha JH, Ham BJ, Han CS, et al. Association between norepinephrine transporter gene polymorphism and major depression. Neuropsychobiology 2004;49(4):174-7.

244. Hattori E, Yamada K, Ebihara M, Toyota T, Nankai M, Shibuya H, et al. Association study of the short tandem repeat in the 5' upstream region of the cholecystokinin gene with mood disorders in the Japanese population. Am J Med Genet 2002;114(5):523-6.

245. Pauls J, Bandelow B, Ruther E, Kornhuber J. Polymorphism of the gene of angiotensin converting enzyme: lack of association with mood disorder. J Neural Transm 2000;107(11):1361-6.

246. Furlong RA, Keramatipour M, Ho LW, Rubinsztein JS, Michael A, Walsh C, et al. No association of an insertion/deletion polymorphism in the angiotensin I converting enzyme gene with bipolar or unipolar affective disorders. Am J Med Genet 2000;96(6):733-5.

247. Arinami T, Li L, Mitsushio H, Itokawa M, Hamaguchi H, Toru M. An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders. Biol Psychiatry 1996;40(11):1122-7.

248. Jahnes E, Muller DJ, Schulze TG, Windemuth C, Cichon S, Ohlraun S, et al. Association study between two variants in the DOPA decarboxylase gene in bipolar and unipolar affective disorder. Am J Med Genet 2002;114(5):519-22.

249. Jun TY, Pae CU, Chae JH, Bahk WM, Kim KS. Polymorphism of CTLA-4 gene for major depression in the Korean population. Psychiatry Clin Neurosci 2001;55(5):533-7.

250. Furlong RA, Ho LW, Rubinsztein JS, Michael A, Walsh C, Paykel ES, et al. A rare coding variant within the wolframin gene in bipolar and unipolar affective disorder cases. Neurosci Lett 1999;277(2):123-6.

251. Zubenko GS, Hughes HB, Stiffler JS, Zubenko WN, Kaplan BB. Genome survey for susceptibility loci for recurrent, early-onset major depression: results at 10cM resolution. Am J Med Genet 2002;114(4):413-22.

2 RESEARCH AIMS AND METHODS

2.1 RATIONALE OF PROPOSED STUDY

There are relatively few published studies at the population level using the Rome II criteria for IBS and it is unknown whether the risk factor associations of Rome II defined IBS are the same as those of Rome I or Manning defined IBS. Additionally, since there is evidence of substantial overlap between IBS and MDD, it is thought that MDD may demonstrate a significant association with IBS. Despite knowledge of the overlap between these two disorders, little is known about the etiology of their co-occurrence. Although both IBS and MDD have demonstrated familial transmission, associations with serotonin, and similar epidemiologic risk factors, not much is known about the heritability, familiality, or biologic mechanism of their co-occurrence. Thus this study will examine whether a common familial biologic mechanism (genetic or environmental) may be underlying the co-occurrence of IBS and MDD. Results from this study would be a first step in demonstrating a need for further research into biologic mechanisms responsible for a demonstrated lifetime co-occurrence. This could include a better understanding of how 5-HT_3 and 5-HT_4 agonists and antagonists work for alleviating IBS symptoms. Additionally, a better understanding of their

biology could lead to investigation of the use of other serotonin and antidepressant drugs for the treatment of IBS. Further, if in fact these disorders do share some common biologic mechanism, it is possible that eventually a new drug could be developed and tested specifically for those individuals with both IBS and MDD. Aside from the implications for medications, increased knowledge about the biology of their co-occurrence would also be the background for the justification of candidate gene studies in IBS and in those with IBS and MDD. Moreover, our results will be important for future research, as statistical genetic models incorporating these interactions should better approximate the biological reality of these traits, and make it easier to detect, localize, and identify genes contributing to the covariation of MDD and IBS, two common co-morbid disorders of public health burden and significance.

2.2 SPECIFIC AIMS

Aim 1: Estimate the prevalence of Rome II IBS in various twin populations.

1. Evaluate Rome II IBS prevalence in two different populations: one US twin study and one Swedish twin study.

2. Determine prevalence of Rome II IBS in subgroups based on age, gender, co-morbid disorders, and family history.

Aim 2: Describe the personal and disease history associations of Rome II IBS in the two population-based twin samples.

1. Evaluate association between personal risk factors and IBS, specifically gender, self-rated health, and BMI.

2. Evaluate association between specific diseases and IBS, such as lifetime MDD, chronic widespread pain, and chronic fatigue.

Aim 3: Estimate the contributions of genetic and environmental sources of variation in liability to co-morbid IBS and MDD.

1. Evaluate association between IBS and zygosity.

2. Assess significance of genetic, shared environment, and unique environment in lifetime co-morbid IBS and MDD.

2.3 MATERIALS AND METHODS

2.3.1 Reliability and Validity of Questionnaire Instruments

A. Irritable bowel syndrome (IBS)

All of the aforementioned criteria for IBS (Manning, Rome I, and Rome II) are based on symptomatology (1). At this time, no validation studies of the Rome II criteria for IBS have been published, although a few are in progress (personal communication with DA Drossman on 10/21/2003). However, by examining the history and evolution of the symptom criteria, from Manning to Rome I and then Rome II, the validity of IBS by Rome II criteria is implied.

Beginning with the Manning criteria for IBS, two factor analyses of the criteria were completed (2, 3). Results of both these studies demonstrated an IBS factor that was defined by only three of the Manning criteria. These studies helped to refine the criteria for IBS and have subsequently been incorporated into both the Rome I and the Rome II criteria. The three factors identified were relief of pain with defecation, loose stools with onset of pain, and more frequent bowel movements with the onset of pain (2, 3). Both of these studies used participant administered questionnaires to obtain information on bowel symptoms. The Rome I criteria were established as a refined subset of the Manning criteria. Another factor analysis was completed using the Rome I criteria (4). This study mailed the Bowel Disease

Questionnaire (BDQ) to individuals in the USA, Australia, Germany, and Sweden. A different validated questionnaire was used in Sweden, however it was considered to be comparable to the BDQ. In this factor analysis, an IBS factor was demonstrated in all four countries, which suggests that the patterns of GI symptoms and groupings of individuals are similar across these cultures. The common items that significantly defined the IBS factor in all four countries were: lower abdominal pain, pain relieved by a bowel movement, more bowel movements with pain, and looser stools with pain (4), which happen to form the core of the Rome I (and II) criteria for IBS.

Using the clinician's diagnosis of IBS as a gold standard, a study of the predictive value of the Rome I criteria was completed using both a prospective component and a retrospective component. Both the prospective and retrospective components of the study were completed in patients referred to gastroenterology clinics and obtained all necessary information from medical record reviews. In the retrospective analysis, the sensitivity of the Rome I criteria and the absence of red flags was 65%, specificity of 100%, and positive predictive value of 100%. Additionally, at the end of 2 years of follow-up none of the patients diagnosed with IBS had their diagnosis changed. The prospective analysis had a positive predictive value of 98%. Taken together, these results suggest that the Rome I criteria for IBS combined with a lack of red flags correspond to an IBS diagnosis (5).

Preliminary results from one of the Rome II validation studies that is currently in progress demonstrated that patient responses to a questionnaire would correctly identify 58% of IBS patients in primary care clinics, and 66% in gastroenterology clinics (6). A drawback of the Rome II criteria demonstrated in this study was that while Rome II identified more IBS cases than were diagnosed by a physician using the Rome I criteria, the Rome II criteria were not as accurate as the Rome I criteria in diagnosing patients with an alternative clinical diagnosis (6). Thus the Rome II criteria may be problematic for other diagnosing GI disorders in gastrointestinal studies that are not examining IBS.

Since the Rome II criteria are generally accepted by researchers in IBS, assessment by self-report began. The use of self-report questionnaires to assess gastrointestinal symptoms is available. Liebbrand and colleagues created the Gastro-Questionnaire for the screening and psychometric measurement of functional gastrointestinal disorders (7). The reproducibility and validity of this instrument was performed in Germany on an unselected sample of the population, and thus may not be representative of the general German population. Nonetheless, the results from this study demonstrated good reliability and good internal consistency (Cronbach's alpha for frequency and severity items were 0.86 and 0.87, respectively). The factor analysis performed explained 60.7% of the variance and obtained a six-factor solution. Based on this study, the Gastro-Questionnaire is thought to be a reliable and

valid instrument for the assessment of functional gastrointestinal disorders (7).

In 2003, the self-administered Biliary Symptom Questionnaire (BSQ) and the shortened version (sBSQ) were created and assessed (8). Reproducibility of the instrument was assessed using the test-retest method, and median kappa values were 0.65. Comparing BSQ responses with symptoms obtained by structured interview assessed concurrent validity. The median kappa value for concurrent validity was 0.61. Discriminant validity compared the BSQ-based diagnoses of biliary symptoms, gastroesophageal reflux disease (GERD), and IBS with patients' final diagnoses. Using the BSQ responses, investigators distinguished IBS and GERD 79% and 90% of the time, respectively. Therefore, for biliary symptoms, the BSQ is reproducible, and has good concurrent and discriminant validity (8).

Specifically examining the IBS subsection of the BSQ, the reproducibility has a kappa of 0.65, with 84% agreement. The kappa for the concurrent validity is 0.63 with 79% agreement. Additionally, when the BSQ score was compared with the diagnosis by a clinical gastroenterologist, the kappa value was 0.51 with 78.9% agreement. Overall, the BSQ performed well and thus is shown to be reproducible, with concurrent and discriminant validity for IBS (8).

Based on results of these prior studies, the IBS component of the Adult Health and Personality (AHP) Survey questionnaire uses the Rome II research diagnostic criteria for IBS, adapted to a self-report format. Responses to questions were then converted into a dichotomized response (IBS yes or no) using the algorithm for the research diagnostic criteria of the Rome II committee (1). This was thought to be a valid assessment of IBS status as it is similar to the BSQ. The Screening Across the Lifespan Twin (SALT) survey does not contain these questions. As such, a concordance between the AHP survey and the SALT survey questions was determined.

Concordance of IBS definition. Questions obtaining information on IBS in the SALT study were mapped onto appropriate questions dealing with IBS in the AHP study, and corresponding kappa statistics were determined by applying the AHP study's Rome II IBS definition and the SALT-mapped IBS definition to the entire AHP sample. By doing this, it was shown that there was good agreement (Cohen's kappa=0.85-0.92) between a subset of questions on the SALT study, and the Rome II definition of IBS obtained in the AHP study. Additionally, only 18 IBS cases out of the 203 total were misclassified during this procedure. Therefore, I used the subset of questions that led to this concordance to define IBS in the SALT study. This choice was made to make results from both studies comparable due to the use of the same (statistically speaking) definition of IBS in both studies.

B. Major Depressive Disorder (MDD)

The diagnosis of major depression is a subjective diagnosis by the physician, as no biological test is available to determine whether a person definitely has the disorder. With that caveat in mind, a physician diagnosis of major depression according to DSM criteria is considered the gold standard (9). An instrument has been developed to diagnose and differentiate psychiatric disorders (10). This instrument is the Structured Clinical Interview for DSM-III-R (SCID) (11, 12), and the revised SCID for the updated DSM-IV criteria. This proposal uses self-report of MDD symptoms that is based on the SCID for DSM-IV diagnoses.

The purpose of the SCID was to be able to extract all information necessary for a psychiatric diagnosis through the use of trained interviewers, not necessarily physicians (12). The SCID was organized to systematically examine the DSM-III-R criteria, and the subsequent DSM-IV criteria, for each psychiatric disorder in a systematic approach (10). It has a modular structure and approximates the decision tree approach of the DSM-III-R criteria (10, 11, 13). Using the modular approach, only certain sections are necessary for certain disorders, and as such, it can be adapted based on which psychiatric disorder diagnosis is of interest in a particular study (10, 11). Additionally, by using the decision tree approach, the interviewer can skip modules based on the patients responses to key questions, which are considered essential criteria for a diagnosis in that module (10, 11).

The SCID is a semi-structured diagnostic interview (10, 11). Its structure is such that it can systematically cover all psychiatric diagnoses in one interview. It is considered semi-structured as opposed to fully structured because the trained interviewers are allowed to aide in the diagnosis by incorporating not only verbal responses, but also behaviors demonstrated during the interview, non-verbal actions seen during the face-to-face interview, and the patient's documented medical history (12). Additionally, if answers to questions are unclear or indeterminant, the interviewer is allowed to probe the participant with their own questions until enough information has been obtained to make a diagnostic decision (12). The SCID was designed for use as a face-to-face interview, with the interviewer being someone with clinical experience and knowledge of psychopathology and psychiatric diagnosis, so as to conduct a diagnostic interview without an interview guide (11). Most studies have trained individuals with professional backgrounds in psychiatry, psychology, or social work to be able to perform the SCID interview and obtain reliable diagnoses from its use (11).

The reliability and validity of the SCID is important, as the basis of the MDD definition used in the Adult Health and Personality (AHP) survey is from an instrument similar to the SCID that is modified to a self-report format. Reliability of psychiatric diagnoses obtained through a SCID interview have been assessed in previous studies (10, 12-14). In the reliability study of the SCID for all psychiatric disorders by Williams, et al., the test-retest reliability

method was used in four patient and two non-patient sites in the US, and one patient site in Germany (14). In this reliability testing method, two interviewers, all with mental health training (either Masters, PhD, or MD), independently interview and diagnose the same subject. Diagnoses are then compared to determine the reliability of the instrument. In this study, the amount of information available to the interviewers was limited to a summary of the hospital admission evaluation and the interviewers were not allowed to consult with the patients full medical record, or full hospitalization file (14). Five hundred ninety two subjects were included in this study. The results indicate a fair agreement in the use of the SCID for major depression in the patient sample, with kappa statistics ranging from 0.37 to 0.82 for current depression and from 0.53 to 0.80 for lifetime depression (14). For both of these ranges, it must be noted that the lowest kappa values are from the population of patients that are in a substance abuse treatment unit, which may impose additional uncertainty in the use of the SCID (15), as a 20-30% correlation between MDD and substance abuse disorders has been reported (16). Without the results from the substance abuse treatment unit, the ranges for the kappa values change to 0.54-0.82 for current depression, and 0.61-0.80 for lifetime depression, all demonstrating moderate to good agreement (14). In the non-patient samples, the SCID performed poorly, with an overall study kappa of 0.42 for current depression, and 0.49 for lifetime depression. These results are not that encouraging when presented by themselves.

However, when compared to other diagnostic instruments for psychiatry, such as the diagnostic interview schedule (DIS) which has a kappa for lifetime major depression of 0.61, and the composite international diagnostic interview (CIDI) which has a kappa of 0.66 for lifetime major depression and 0.52 for current lifetime depression, the results of the SCID appear similar. In regard to the SCID and the DIS, the results from the SCID are very encouraging, especially since the DIS is a fully structured interview, where the only variability in a test-retest reliability study is when a patient responds yes to one interviewer and no to the other interviewer for the same question (14). Using the SCID, the variation comes from differences in interviewing style, depth of probing, and clinical judgment (14), so while there is more room for variability and thereby a lower kappa value, it appears to perform similarly to a structured interview where there is less variability.

In the reliability study by Skre, et al., a different method of assessing reliability was used. Here, each interview using the SCID was audiotaped, and two additional raters besides the interviewer determined a diagnosis based on that interview. Thus, the kappa statistics in this study measured the inter-rater agreement (12). This study was specifically examining the reliability of the SCID for major depression, and the interviewers were a medical doctor, psychologist, and psychiatry graduate students (12). For this study both current and lifetime depressions were grouped together. The resulting kappa value for inter-rater agreement for major depressive disorder

was 0.93, indicating excellent agreement (12). Thus, both the Williams and Skre studies demonstrate acceptable reliability for the diagnosis of major depression using the SCID.

Riskind et al., performed another reliability study on the SCID (10). In this study, interviews of 75 psychiatric patients were videotaped and two independent diagnoses were made, again testing inter-rater agreement. In this study, the kappa statistic value was 0.72 for major depressive disorder, however MDD was not separated into current or lifetime depression. Additionally, the kappa statistic is associated with an 82% agreement rate between interviewers in the study. Thus this study demonstrated that there is satisfactory diagnostic reliability amongst interviewers who are trained to use the SCID (10).

Results from the Williams, Skre, and Riskind studies demonstrate adequate reliability of the SCID for diagnosing major depression. Moreover, both inter-rater agreement and test-retest agreement were satisfactory. Therefore, the SCID is a reliable and valid instrument to diagnose major depression, higher among those diagnosed with MDD than non-patient samples. The studies used in this proposal are non-patient samples, and thus lower kappa statistics for reliability should apply to these data.

Since the development and validation of the SCID, researchers became interested in the use of self-reported depression questionnaires in research. One such self-report questionnaire is the Inventory to Diagnose Depression (IDD) which was designed as a self-report scale to diagnose MDD based on DSM-III criteria (17). Scoring was completed by summing the scores for each question based on a predetermined value; a diagnosis was determined when the summation surpassed a predetermined critical value (17).

In an inpatient population, the IDD was shown to have high test retest reliability and high internal consistency. The diagnostic agreement between the IDD and a clinician diagnosis of MDD was as high as that demonstrated in studies examining the inter-rater reliability of a diagnosis of MDD (17). In this study, the kappa values for the IDD were 0.66 and 0.58, for symptoms only and symptoms plus duration of symptoms for MDD, respectively. Thus, the IDD demonstrated good agreement with clinical diagnosis (17).

The IDD has also shown good discriminant validity (18). Uehara et al. demonstrated that the IDD was able to differentiate between individuals with MDD, individuals with anxiety disorders, and general controls with a concordance between the IDD and clinical diagnosis of 0.81. Thus the IDD is able to clinically distinguish individuals with MDD from individuals with other mood disorders (18).

When the IDD was used in a population of alcohol-dependent men and women, the overall agreement of the IDD to the SCID was good (kappa=0.66) (15). However, the agreement changed based on whether the responders were alcohol abstainers (κ=0.78) or recent alcohol consumers (κ=0.55) (15). Therefore, the IDD should be used with caution in a population of recent alcohol consumers.

Another self-report instrument for depression is the Short Depression Interview (SDI) (19). The diagnosis of depression using this instrument is based on the DSM-IV criteria. Using this instrument in general practitioners clinics, the reproducibility of a major depression diagnosis was good, with a kappa of 0.63 (19). The validity of major depression as a diagnosis as assessed by general practitioner compared to self-report was demonstrated to be satisfactory (r=0.35-0.61). However the correlation coefficient for the test retest of the number of depressive symptoms was 0.82, and in 75% of patients the test retest difference in symptom number did not exceed one symptom (19). Based on these results, the SDI has moderate to good agreement with general practitioner diagnoses of major depressive disorder.

The Diagnostic Inventory for Depression (DID) is a self-report questionnaire that diagnoses depression based on the DSM-IV criteria (20). This instrument was compared with three other validated self-report questionnaires, the Beck Depression Inventory (BDI), the Hamilton Rating

Scale for depression, and the Clinical Global Index of Depression Severity. The correlation between the DID and each of these instruments was 0.83, 0.73, and 0.73, respectively. Additionally, it was shown that the threshold scoring used in the DID performed comparably to the DSM-IV based algorithmic approach in identifying cases of depression. The DID also performed as well as the BDI in identifying SCID diagnosed cases of current depression (20). These results demonstrate that the DID is another validated and reliable method of obtaining a DSM major depression diagnosis using self-report.

As these studies have demonstrated, reliable and valid instruments exist for the self-report of depression in certain populations, such as inpatients, general practitioners clinics, and alcohol-consumers. The concordance with the SCID is good, and the SCID has also been shown to be a valid and reliable instrument for diagnosing depression when compared with a physician diagnosis. Thus the use of self-report for depression is a good estimate of a physician diagnosis of major depressive disorder. Indeed Tondo et al. demonstrated that patients tend to rate their symptoms of depression to be worse than their physicians' assessment when both patient and physician use their respective forms of the Inventory for Depressive Symptomatology (IDS) (21). Additionally, self-report of depression has yielded similar lifetime prevalences, gender ratios, and heritability estimates as direct interview studies (22, 23).

C. Self-Rated Health

The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) has been used to determine a person's summary physical health and summary mental health scores (24), and is used as a quality of life indicator. The SF-36 is a shortened version of the 149 health status questions developed as a potential tool for monitoring patient outcomes in a clinical setting (25). Three aspects of health are covered in the SF-36; functional status, well-being, and overall evaluation of health using eight separate scales (physical functioning, social functioning, role limitations attributable to physical limitations, role limitations attributable to emotional problems, mental health, energy and fatigue, pain, and general health perception) (25-27). This questionnaire has been validated and shown to be reproducible (24). Results from studies performed in different populations have consistently demonstrated internal consistency of the SF-36 with corresponding Cronbach's α coefficients ranging from 0.76-0.93 (25-27). Two of these studies also evaluated the test-retest reliability of the SF-36, and it received acceptable pearson correlation coefficients, ranging from 0.60-0.89 (25, 26). Another study examined criterion validity of the SF-36 by comparing scores for the seven multi-item dimensions assessing functional status and well-being with a single global health question and the results strongly supported a linear trend of decreasing SF-36 score corresponding to worse reported health on the initial question (27). Taken together, these results suggest that

the SF-36 is reliable to monitor health. However, due to economic considerations, space considerations on questionnaires, as well as the amount of time it takes for individuals to complete the questions (24), the SF-36 has been condensed into a 12-Item Short-Form Health Survey (SF-12) (24).

The SF-12 has been validated against the SF-36, and has demonstrated a multiple R^2 of 0.911 in the prediction of the SF-36 physical component summary score, and 0.918 in the prediction of the SF-36 mental component summary score in the general US population (24). Therefore the questions contained on the SF-12 were the best predictors of both scales on the SF-36. The SF-12 physical and mental component scores also were highly correlated to the corresponding SF-36 physical and mental component scores (r=0.951 and 0.969, respectively) (24). Additionally, independence between the physical and mental components was maintained, as the two components were very weakly correlated with each other (r=0.06) (24). Test retest reliability of the physical and mental components of the SF-12 yielded coefficients of 0.890 and 0.760, respectively, in a US sample (24). These coefficients compare favorably with those for the corresponding SF-36 components. All of these statistics lead to the conclusion that the SF-12 is able to maintain satisfactory reliability to the SF-36 while reducing the number of items in the questionnaire (24).

The AHP questionnaire included a section of questions on self-rated health. This section consisted of 12 questions, namely the questions that comprise the Medical Outcomes Study 12-Item Short Form Health Survey (SF-12) (24). As was discussed previously, this instrument was found to be valid and reliable in other US studies.

2.3.2 Description of Study Populations

In order to address the aims of this proposal, two studies were used. The first study, the Adult Health and Personality (AHP) Survey, is a subset of the Mid-Atlantic Twin Registry (MATR). This study was used to investigate aims 1 and 2. The second study, the Screening Across the Lifespan Twin Study (SALT) study, is a subset of the Swedish Twin Registry (STR). This study was used to primarily investigate aim 3, however aims 1 and 2 were also investigated but not incorporated into a manuscript due to negotiations with the STR.

A. Parent Study: Mid-Atlantic Twin Registry (MATR)

The Mid-Atlantic Twin Registry is a population-based registry of twins of all ages who are born in or live in North Carolina, South Carolina, and Virginia, and who are willing to consider participating in health-related research. There are more than 51,000 twins in the registry. Complete twin pairs are responsible for roughly 46,000 of the twins in the registry. The identification of twins and their families is done by using publicly available

birth records, public driver's license information, and through public and private school records, which are made available only for the purpose of medical research. Scientists connected to the MATR have explored such topics as epilepsy, cardiovascular disease, diabetes, cancer, obesity, inflammatory bowel disease, attention-deficit hyperactivity disorder, clinical depression, anxiety, pregnancy complications, periodontal disease, alcohol, nicotine and other drug abuse, stress and coping, religiosity and spirituality, social support, and parenting styles (28).

B. Adult Health and Personality (AHP) Survey

Data collection. Approximately 16,000 twins participating in the MATR were initially mailed a self-report questionnaire. If the questionnaire was not returned within five weeks, a reminder was sent to the same address. An attempt was also made to telephone non-responders if there was no response to the reminder mailing. The initial mailing was sent out in late 2001, and all data collection was complete by early 2002.

***Response rate*.** Completed questionnaires were returned by 4,591 twins. Response rate for this survey is not known due to not knowing who in the MATR was actually mailed a survey. Therefore, the denominator is not enumerated, but it is estimated that 30% of individuals responded, which is thought to be an underestimate of the true response rate, as 10-25% of the mailed questionnaires never reached potential participants due to incorrect

mailing addresses, incorrect forwarding of mail, etc. Zygosity was determined for 79% of these respondents. This represents 3764 twins, 988 (26.3%) monozygotic, 1985 (52.7%) dizygotic, and 791 (21.0%) twins of unknown zygosity.

Questionnaire. The information collected in this questionnaire dealt with personal history of many chronic diseases, current status of some disorders, limited information on family history for an assortment of diseases, personality disorders, and behaviors related to smoking and exercise. The variables that were used in this analysis are age, gender, calculated body mass index (BMI, which is kg/m^2), mental health standardized score on the SF-12, physical health standardized score on the SF-12, chronic fatigue syndrome-like illness (a surrogate for chronic fatigue syndrome, as all that is missing for a diagnosis is the physical exam), chronic widespread pain (a surrogate for fibromyalgia, as all that is missing for a diagnosis is the physical exam), Rome II irritable bowel syndrome, DSM-IV major depression, and self history of IBS, Crohn's disease, and ulcerative colitis. These variables were chosen based on prior literature that stated an association of these variables with either IBS or MDD. The directed acyclic graph for this analysis is shown in Figure 2.1.

Working definition of IBS. The questionnaire contained items that followed the research diagnostic criteria of the Rome II criteria for an IBS

diagnosis (1) , and the accompanying diagnostic algorithms for IBS are applied to the questionnaire responses to obtain an IBS diagnosis. The Rome II criteria were decided upon through consensus by gastroenterologists and are used in research in order to be able to compare results (1). For the analysis of the AHP study, any twin that fulfilled the Rome II criteria based on the questionnaire responses and did not report a self-history of Crohn's disease or ulcerative colitis were considered an IBS case. Those who did not fulfill the Rome II criteria were the non-IBS controls.

***Working definition of MDD*.** Major depressive disorder (MDD) was determined based on the guidelines in the Diagnostic and Statistical Manual, 4th edition (DSM-IV) (29). This determination is through responses to a self-administered depression screener containing expanded versions of the section of the SCID interview for MDD, adapted to a self-report format (23). The DSM-IV criteria were then applied to questionnaire responses by a computer algorithm to assess the presence of MDD during any two week period over the participants' lifetime (lifetime MDD).

C. Parent Study: Swedish Twin Registry (STR)

The Swedish Twin Registry was established in 1961 and is a research resource maintained at the Karolinska Institutet. The Swedish Twin Registry (http://www.mep.ki.se/twinreg/index_en.html) includes more than 140,000 twins, thus making it the largest registry of twins in the world. It was

established in the 1950's to study the risk factors for cancer and cardiovascular diseases, while controlling for genetic risk of disease. The registry is updated monthly through linkages to three national registries: the address registry, the cancer registry, and the cause of death registry (30, 31).

There are three age cohorts in the registry: the older cohort, comprised of 10,945 pairs of like-sexed twins born 1886 through 1925; the middle cohort, comprised of approximately 50,000 pairs of like- and unlike sexed twins born 1926 through 1967; and the younger cohort, twin pairs born since 1967 which number roughly 25,000. Questionnaire data has been collected from members of the old cohort in 1961, 1963, 1967, and 1970, and from like-sexed members (born 1926-1958) of the middle cohort in 1973. The questionnaire data include items about health (cardiovascular disease, cancer, asthma), health-related behaviors (tobacco, alcohol, and caffeine consumption), personality, physical activity, eating habits, and environmental stressors (30).

D. Screening Across the Lifespan Twin (SALT) Study

***Data collection*.** Twins born in Sweden in 1958 or earlier were included. A computer assisted telephone interview about different diseases and symptoms was used. In order to minimize the risk of bias due to potential age effects, an attempt was made to interview both members of a twin pair within a month of each other.

Following the pilot study that was completed during the fall of 1996 and spring of 1997, full-scale data collection began in March 1998. The telephone interviews were completed by trained interviewers, with adequate medical background, using a computer-based data collection system. The interview structure began with an introductory section containing questions about birth order and weight, validated zygosity questions, contact with twin partner and family. Once those were completed, a checklist of common illnesses, prescription and nonprescription medication use, and permission to collect medical records was asked for. Basic demographic and social data were obtained, including occupation, education, consumption of alcohol, tobacco, and caffeine. If it was determined that the twin could not be interviewed, the interview was conducted with an informant (32).

***Questionnaire*.** The portion of the interview concerned with common illnesses was designed to obtain as much information as necessary to screen for most common complex diseases, including obesity and eating disorders, heart and vascular diseases, diabetes, women's health, asthma, allergy, eczema, headache, gastrointestinal problems, arthritis, osteoporosis, muscle pain, sleep problems, chronic fatigue syndrome, Parkinson's disease, depression, anxiety, mania, epilepsy, smoking, coffee, and alcohol habits, fibromyalgia, and irritable bowel syndrome (32). Emphasis was put on diagnostic items rather than just asking a twin whether they have a disease. These diagnostic items were presented in a branching format so that within a

disease area follow-up items were asked only if the participant responded positively to the key introductory items. These items were compiled by experts in each of the disease areas (32, 33). If standardized instruments were available, such as the short Computerized International Diagnostic Interview (CIDI) for psychiatric disorders (34), these were used.

***Working definition of IBS*.** The diagnostic algorithm used to diagnose IBS was not an exact match of the Rome II criteria. Therefore, using exact Rome II criteria for the analysis in this study was not possible because the questions were not specific enough to fulfill the Rome II criteria. In an effort to allow this study and the AHP study to have similar definitions of IBS, a concordance between the questions pertaining to abdominal problems in the SALT study, and the Rome II questions of the AHP study was undertaken as described on page 150. Based on this analysis, the questions from the SALT questionnaire that best correspond to the Rome II criteria were used for a diagnosis of IBS. The IBS definition was comprised of positive responses to the following questions: recurrent abdominal discomfort, abdominal discomfort that lasted as least 7 days a month, if intestinal problems were more prominent when feces became looser and defecation more frequent, and reporting recurrent problems with pain in either the upper abdomen, lower abdomen, or another part of the abdomen. Subjects reporting a history of Crohn's disease (n=34), ulcerative colitis (n=55), stomach ulcers (n=59), intestinal ulcers (n=46), or any combination of these

(n=52) were excluded. Together, the subset of questions combined with the exclusions was applied to the cohort to identify Rome II IBS cases in the SALT study. By employing this method of definition in the SALT study, it was thought that the results would be more readily comparable due to the use of the same (statistically speaking) definition of IBS in both studies.

***Working definition of MDD*.** This determination was done through an algorithm using the participant's answers to the short form of the computerized international diagnostic interview (CIDI) which was obtained through a telephone interview, completed by trained medical interviewers (33).

2.3.3 Data Analysis

Due to the breadth of information available from the interview performed in the SALT study, it is safe to assume that variables included in the AHP study were also available in the SALT study. Additionally, since this questionnaire is funded through the Karolinska Institutet, the variables that I had access to were limited due to the permission that I was given to use this data. Thus, the same variables that were used to analyze the AHP study were also used in the SALT study. In this way, I was able to compare results of the prevalence and associations of Rome II IBS, and additionally was able to perform a co-twin control analysis using the SALT study due to near complete zygosity determination for the entire sample.

A. Outcome of Interest

The outcome of interest was Rome II defined IBS. This outcome was dichotomous, that is either IBS was present or absent. In both studies, this variable was derived by utilizing the responses to the corresponding gastrointestinal questions, excluding those with Crohn's disease and ulcerative colitis, and translating them into a Rome II IBS diagnosis based on the algorithm defined by the Rome committee (1).

B. Exposure of Interest

For aims 1 and 2, the exposure of interest varied depending on which previously reported covariate was investigated in relation to its association with Rome II defined IBS. Thus, the exposures were gender, lifetime major depression, chronic widespread pain, chronic fatigue syndrome-like illness, mental and physical scores of the SF-12, body mass index, and age. For aim 3, lifetime major depression was the main exposure of interest for the co-twin control analysis of Rome II IBS.

C. Statistical Analysis Related to Specific Aims

Aim 1: Estimate the prevalence of Rome II IBS in various twin populations.

In order to describe the prevalence of IBS, basic descriptive statistics were calculated for each study. The SAS program, version 8.02, (35) was used to obtain the appropriate frequencies. Of particular interest was the

frequency of Rome II defined IBS in the whole sample, as well as stratified by gender (Tables 2.2, 2.3, 2.31, 2.32, 2.38, and 2.39).

Bivariate frequencies were then calculated with those covariates thought to influence IBS (36). Variables of interest were gender, age, lifetime MDD, chronic fatigue syndrome-like illness (all criteria but physical exam needed for chronic fatigue syndrome) (37), chronic widespread pain (measure of fibromyalgia), SF-12 (mental and physical components), and body mass index.

Aim 2: Describe the personal and disease history associations with Rome II IBS in the two population-based twin samples.

To assess the personal and disease history associations with Rome II IBS, generalized estimating equations were used in the SAS software (35) in order to adjust for the clustered nature of the twin data while determining the relevant associations with IBS among the previous covariates that have demonstrated an association with IBS. A crude bivariate analysis of IBS (yes/no) and each exposure mentioned above, including estimates of the odds ratios and corresponding 95% confidence intervals were obtained (36, 38) (Tables 2.5, 2.6, 2.33, 2.34, 2.41, and 2.42). The continuous variables were age, BMI, SF-12 mental health score, and SF-12 physical health score. BMI and the SF-12 scores were also analyzed as dichotomous variables (above/below the BMI for obese, and for SF-12 scores, as above/below the

population mean score), and as quartiles. Based on previous research, the directed acyclic graphs (DAGs) in Figures 2.1-2.8 were used to aid in determining the appropriate confounders and covariates to be included in the modeling of IBS which was incorporated into the modeling strategy of Kleinbaum (39). The covariates of interest are presented in Table 2.1 as well as a description of the variables with their potential coding schemes.

To use DAGs to determine which covariates should be included in the multivariable models, a graph (Figures 2.1-2.8) was drawn to visually depict the causal associations between exposure, outcome, and covariates (40, 41). The arrows on the graph signify associations, and the association of interest was depicted by a question mark above that arrow (41, 42). Graphical rules of analysis were then used on these graphs to determine which variables were potential confounders and needed to be considered as such during the multivariable modeling (40).

Stratification analysis (effect measure modification assessment). For the remaining analyses of this aim, lifetime major depression is used as the main exposure to illustrate the process of assessing effect measure modification that was conducted for several other covariates, including BMI, chronic widespread pain, chronic fatigue syndrome-like illness, SF-12 physical and mental health score, gender, and age. This further elucidated the relevant associations with Rome II defined IBS.

Effect measure modification (EMM) was assessed on both the additive and multiplicative scales. All assessments were completed prior to confounding assessment. To determine which covariates were effect modifiers, crude and adjusted odds ratios (ORs), corresponding 95% confidence intervals (CIs), and Breslow-Day p-value were calculated. The crude estimate was obtained from the bivariate analysis of lifetime MDD and IBS result. Covariates were considered as strong potential effect modifiers if the Breslow-Day p-value was < 0.20, as possible effect modifiers if the Breslow-Day p-value was between 0.20 and 0.5, and as unlikely effect modifiers if the Breslow-Day p-value was > 0.5. Tables 2.7, 2.10, 2.13, 2.16, 2.19, 2.22, 2.25, 2.28, 2.35, and 2.43 provide a summary of the evaluation of effect measure modification when each covariate was used as the main exposure. To determine appropriate reference groups and to further examine which variables should be kept as potential effect measure modifiers, analyses of joint effects on both the additive and multiplicative scale were conducted as necessary (39).

On the additive scale, the estimate of the association between MDD and IBS was stratified by levels of the potential effect modifier. Stratified odds ratios, 95% confidence intervals, and the interaction contrast ratio (ICR) were calculated. If the ICR is 1, EMM was not considered to be present for that potential effect modifier. An ICR less than 1.0 was suggestive of the

antagonistic EMM, and an ICR greater than 1 suggested synergistic EMM (36).

For assessment of EMM on the multiplicative scale, logistic regression models of the association between MDD and IBS with and without interaction terms between MDD and the potential effect modifiers were compared using the likelihood ratio test. If the likelihood ratio test was significant ($p<0.2$), then the interaction terms modified the association between MDD and IBS. However if the likelihood ratio test was not significant, the interaction term did not modify the association between MDD and IBS.

Confounding assessment. To assess for confounding by those variables found not to be effect modifiers, I incorporated the following three methods: directed acyclic graphs (DAGs), bivariate distribution of the exposure by each covariate among the controls and distribution of IBS by each covariate conditional on non-exposure, and change in the odds ratio. DAGs were drawn based on a review of the literature, and helped to establish potential covariates as confounders. I obtained and compared the odds ratios from the bivariate distribution among controls and among non-exposed for each covariate (Tables 2.8, 2.11, 2.14, 2.17, 2.20, 2.23, 2.26, 2.29, 2.36, and 2.44, depending on the exposure of interest). The change in the estimated odds ratio was obtained by using the following formula: $\ln[OR_{crude}/OR_{adjusted}]$. If the result of this comparison was less then 0.10, the potential confounder of

interest was not a confounder. If the result of this comparison was greater than or equal to 0.10, then the exposure-covariate (E-C) and covariate-disease (C-D) relationships were examined to further assess confounding (37, 39). Additionally, if our DAG illustrated that a covariate was associated with the exposure and related to the outcome, it was included in our model, even if the change in $\ln[OR_{crude}/OR_{adjusted}]$ was less than 0.10. However variables that were affected by the exposure or affected by the outcome were not considered as potential confounders.

In order to examine the E-C relationship, odds ratios were calculated examining lifetime major depression by each of the dichotomous and categorical covariates (i.e. gender, BMI, age, chronic widespread pain, chronic fatigue syndrome-like illness, self-rated mental health score, and self-rated physical health score). All study participants were included in this analysis. Odds ratios (ORs) and 95% confidence intervals were calculated, as was a precision estimate (upper confidence limit divided by lower confidence limit) (43). Tables 2.8, 2.11, 2.14, 2.17, 2.20, 2.23, 2.26, 2.29, 2.36, and 2.44 summarize these results.

The covariate-disease (C-D) relationship was examined by calculating odds ratios of the main outcome (IBS) among individuals in the control exposure group (no lifetime MDD) by each of the dichotomous and categorical covariates (i.e. gender, BMI, age, chronic widespread pain,

chronic fatigue syndrome-like illness, self-rated mental health score, and self-rated physical health score). Odds ratios (ORs) and 95% confidence intervals were calculated, as was a precision estimate (upper confidence limit / lower confidence limit) (43). Tables 2.8, 2.11, 2.14, 2.17, 2.20, 2.23, 2.26, 2.29, 2.36, and 2.44 summarize the results of the exposure-covariate and covariate-disease relationships when each covariate was used as the main exposure and the remaining covariates were used as explanatory variables (37, 39). For each variable, the OR and 95% CI was calculated.

***Multivariate analysis*.** The model of interest is specified below.

Logit P(lifetime IBS) =α +β_1(lifetime MDD) +β_2Var1 +β_3Var2 + β_4Var3 +…

Unconditional logistic regression was used to obtain a valid estimate of the lifetime MDD—IBS relationship that accounts for confounding and effect modification. Unconditional logistic regression was chosen since the data was not matched, the dependent variable was dichotomous, and because there were a small number of parameters relative to the number of subjects (37). Additionally, the natural clustering due to the use of twins was accounted for by a clustering variable in generalized estimating equations.

***Modeling strategy*.** The modeling strategy for this analysis is discussed below, and was adapted from Kleinbaum (39). The first step was to specify the variables that were included in the full model. The goal of this

was to determine independent variables that may be meaningful in order to obtain a valid measure of effect. The variables to be considered were selected because they were biologically or clinically relevant to the occurrence of IBS, or because they were determined to be relevant based on a review of the literature. The full model included all the relevant variables. Tables 2.9, 2.12, 2.15, 2.18, 2.21, 2.24, 2.27, 2.30, 2.37, 2.45, 2.46, and 2.47 show the results for the full model where each covariate was the main exposure.

The next step was to make sure that the full model (and all subsequent models during the modeling process) was hierarchically well-formulated. This meant that any variable that was included in an interaction term was also included as a lower-order component variable in the model (39). When this was completed, assessment of interaction began. The first step for interaction assessment was to run two models, the full model and the model with no interaction terms. By doing this, a 'chunk' test was completed to test for overall interaction by using the likelihood ratio test to determine if the interaction terms as a whole can be eliminated from the model (39). An α of 0.20 was used for the likelihood ratio test when comparing models with and without interaction term(s). Thus, if the p-value associated with the likelihood ratio test was less than 0.20, the interaction term(s) that were removed from the model were returned to the model, whereas if the p-value was greater than 0.20, the removed interaction terms were not significant to the model

and remained eliminated from the model (39). If this initial 'chunk' test was not significant then all the interaction terms were eliminated from the model. If however the 'chunk' test was significant, then at least some or all of the interaction terms were significant and further assessment of the interaction terms was performed (39).

If the 'chunk' test was significant, and thus some or all of the interaction terms were relevant to the model, then the backward elimination method was used to examine the interaction terms one at a time in order to eliminate insignificant variables from the model. This began by examining the least significant interaction term in the model (the interaction term that was associated with the largest p-value in the full model). To determine if this term was significant or not, the full model and the model without this interaction term were compared using the likelihood ratio test as explained above. If this interaction term was not significant, it was removed from the model. If the interaction term was significant, it was retained in the model. The remaining interaction terms were assessed in the same manner. Any covariates that were included in interaction terms that remained in the model at the end of this process were not assessed for confounding (39).

Once the assessment of interaction was complete, the assessment of confounding began. For this, the least significant variable that was identified as a potential confounder in the stratified analysis was removed from the

model. In order to determine if the inclusion of this term confounded the association, the adjusted OR was compared to the crude OR. If ln $|OR_{crude}/OR_{adjusted}| > 0.10$, then the variable was kept in the model; otherwise it was eliminated from the model. As stated previously, variables involved in an interaction term were not considered as confounders because they must remain in the model in order for the model to be hierarchically nested, and thus comparable to the previous model. Only those variables that were not included in significant interaction terms were considered when assessing confounding (39). Tables 2.9, 2.12, 2.15, 2.18, 2.21, 2.24, 2.27, 2.30, 2.37, 2.45, 2.46, and 2.47 concisely summarize all of the modeling information using each covariate as the main exposure in its own model. These tables in combination with the precision estimates for the calculated odds ratios for each model were used to decide upon the most appropriate, unbiased, precise, and parsimonious model (39).

Lastly, since the data describe lifetime prevalence of MDD and lifetime IBS with limited information on age of onset for both disorders, no causality was implied. In order to determine if causality influenced the association between lifetime MDD and lifetime IBS, modeling was also performed with lifetime MDD as the dependent variable (outcome) and lifetime IBS as the independent variable (exposure). This was a sensitivity analysis of the implied causality of these models, thereby determining how sensitive the

statistics and associations were to changes in the causality assumption (44). The DAG for this analysis is shown in Figure 2.7.

Aim 3: Estimate the contributions of genetic and environmental sources of variation in liability to co-morbid IBS and MDD.

To assess the genetic and individual-specific environmental sources of variation in lifetime co-morbid IBS and MDD, a co-twin control analysis was performed using the data from the SALT study. This analysis was completed in the SALT study because it had a larger sample size and because the zygosity of the twins was near complete. The zygosity being near complete was very important, as this analysis takes advantage of the fact that monozygotic and dizygotic twins share different degrees of genetic relatedness, and so in order for the analysis to be informative, the zygosity information must be complete. In this study, I used disease discordant twins, that are twin pairs discordant for IBS, and two control groups: external (not related) controls, and internal (co-twin) controls. The first step in this analysis was to assess the association between MDD and IBS. This was essentially a case-control study in which I compared twins diagnosed with IBS with external controls (other twins not related to the index probands), and evaluated the association between MDD and IBS (32, 33). To complete this step, the methods and analysis plan that was outlined for specific aim 2 was employed with the modification of using conditional logistic regression as

these twin pairs were matched by gender and 3-year age band (37) throughout the remaining twin analyses. This determined if there was an association between MDD and IBS in this population, and the magnitude of the association.

I then determined the extent to which this association was influenced by genetic variation and the extent to which it was influenced by the environmental variation beginning in the second step, which controlled for confounding from unmeasured early environment. In this step, the healthy co-twin of the pair (i.e. the twin without IBS) was used as the control for the diseased (IBS) twin. This minimized confounding by differences in unmeasured childhood or adolescent environments because the twin pair shared the same intrauterine environment and was typically reared together in this sample (32, 33). Again, the methods outlined in specific aim 2, modified to use conditional logistic regression due to age and gender matching of the twins, was employed with the caveat of the specific control used for each IBS case. If the association demonstrated in the first step of this process remained after this process, then the association was not influenced by the common environment.

The third step was to control for unmeasured genetic background. During this step of the analysis, only disease discordant monozygotic twin pairs were used. This controlled for potential confounding from genetic

factors as the cases and controls were genetically identical. Therefore, an observed effect was not confounded by genetic predisposition (32). The same methods were used as previously stated, except that the analysis was restricted to disease discordant monozygotic twin pairs for both the cases and the controls, and conditional logistic regression was used since the twin pairs were age and gender matched. Table 2.48 explains how to interpret results from this analysis.

D. Power Calculations

Allowing major depression to be the exposure of interest, the prevalence of this exposure was allowed to vary between 10 and 20% in order to perform power calculations for this cross-sectional study. Additionally, I allowed the outcome (IBS) prevalence to vary between 5 and 10%. EpiSheet by Rothman (45) was used to obtain the power estimates presented in Table 2.49. These calculations were based on having 203 IBS cases, as that was the number of IBS cases in the AHP study, the smaller of the two studies used in these analyses. By examining Table 2.49, assuming to obtain an association with a prevalence odds ratio (POR) of 1.5, the power in the AHP study varied between 59 and 78%, depending on the actual exposure and outcome prevalence in the study. It was thought that the SALT study will be better powered to detect modest effects, as it is a larger study and thus there should be a larger number of IBS cases.

2.3.4 Human Population, Ethical Consideration, and IRB Issues

The research was conducted in accordance with the principles embodied in the Declaration of Helsinki and under the approval of the University of North Carolina School of Public Health Institutional Review Board.

Procedures were implemented to ensure the privacy of the participants. Every precaution to maintain the confidentiality of participants' records was employed. No subjects were identified in any report or publication about this study. Anonymous identification numbers were assigned to all information from the interviews and surveys. Participants were not represented by name in the data files. Access to these names was limited to the principle investigators.

2.4 TABLES

Table 2.1: Description of variables and potential coding for analysis.

Variable	Description	Coding Scheme
Outcome of Interest		
Irritable bowel syndrome (IBS)	Lifetime diagnosis of IBS	1= Have had IBS in lifetime 0= No IBS in lifetime
Exposure of Interest		
Major depressive disorder (MDD)	Have/had an episode of major depression during lifetime	1= Have had MDD in lifetime 0= No MDD in lifetime
Other Covariates		
Gender		1= Female 0= Male
Age	Age when completed questionnaire	Continuous
Body mass index	Calculated, kg/m^2	4= 18.5-24.9 kg/m^2 3=<18.5 kg/m^2 2=25-19.9 kg/m^2 1=≥30 kg/m^2
Chronic fatigue syndrome (CFS)-like illness	Have/had chronic fatigue during lifetime	1= Have had CF in lifetime 0= No CF in lifetime
Chronic Widespread Pain (CWP)	Have/had CWP during lifetime	1= Have had CWP in lifetime 0= No CWP in lifetime
SF-12, Mental health		Continuous
SF-12, Physical health		Continuous
Personal History Variables		
Crohn's Disease	History of yourself having Crohn's disease during lifetime	1= Yes, had Crohn's disease 0= No Crohn's disease
Ulcerative Colitis	History of yourself having Ulcerative Colitis during lifetime	1=Yes, had ulcerative colitis 0=No ulcerative colitis
IBS	History of yourself having IBS during lifetime	1= Yes, had IBS 0= No IBS

Table 2.2: Characteristics of IBS cases and controls, AHP study, 1999.

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
Total	4,548		212	4.7	4,336	95.3	
Age at interview (years)							2.0043 (0.9809)
< 36 years old	1,120	24.63	52	24.53	1,068	24.64	
36-43 years old	1,194	26.26	59	27.83	1,135	26.19	
44-51 years old	1,204	26.48	56	26.42	1,148	26.49	
> 51 years old	1,028	22.61	45	21.23	983	22.68	
Missing	2		0		2		
Mean (SE)			42.77 (0.25)		42.83 (0.16)		0.37 (0.7113)
Sex							
Male	1,609	35.39	46	21.7	1,563	36.06	-3.41 (0.0006)
Female	2,938	64.61	166	78.3	2,772	63.94	
Missing	1		0		1		
MDD							
Yes	861	19.1	69	32.5	792	18.4	-4.83 (<0.0001)
No	3,667	80.9	143	67.5	3,524	81.6	
Missing	20		0		20		
CWP							
Yes	362	7.96	51	24.1	311	7.2	-8.10 (<0.0001)
No	4,183	92.04	161	75.9	4,022	92.8	
Missing	3		0		3		
CFS-like illness							
Yes	163	3.6	30	14.2	133	3.1	-7.47 (<0.0001)
No	4,385	96.4	182	85.8	4,203	96.9	
Missing	0		0		0		

Table 2.2 (Continued): Characteristics of IBS cases and controls, AHP study, 1999.

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
BMI							
≤24.9 kg/m^2	1,983	43.6	80	37.7	1,903	43.9	4.7160 (0.0946)
25-29.9 kg/m^2	1,509	33.2	71	33.5	1,438	33.2	
≥30 kg/m^2	1,056	23.2	61	28.8	995	22.9	
Missing	0		0		0		
Mental Health Score							
< mean	1,395	31.4	100	48.3	1,295	30.6	-5.06 (<0.0001)
≥ mean	3,042	68.6	107	51.7	2,935	69.4	
Missing	111		5		106		
Mean (SE)			47.00 (0.66)		51.02 (0.15)		5.93 (<0.0001)
Physical Health Score							
< mean	1,135	25.6	107	51.7	1,028	24.3	-8.10 (<0.0001)
≥ mean	3,302	74.4	100	48.3	3,202	75.7	
Missing	111		5		106		
Mean (SE)			45.59 (0.63)		51.52 (0.15)		9.19 (<0.0001)

Table 2.3: Characteristics of IBS cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square	Cases		Controls		Chi-Square
	No.	%	No.	%	statistic (p-value)*	No.	%	No.	%	statistic (p-value)*
Total	46	2.9	1,563	97.1		166	5.7	2,772	94.3	
Age at interview (years)					3.8859 (0.6921)					4.3345 (0.8258)
< 36 years old	8	17.4	308	19.7		44	26.5	760	27.4	
36-43 years old	10	21.7	380	24.3		49	29.5	755	27.2	
44-51 years old	13	28.3	447	28.6		43	25.9	701	25.3	
> 51 years old	15	32.6	428	27.4		30	18.1	555	20.0	
Missing	0		0			0		1		
Mean (SE)	44.19 (0.27)		44.38 (0.25)		1.80 (0.0740)	41.98 (0.20)		41.96 (0.19)		-0.45 (0.6502)
MDD										
Yes	9	19.6	213	13.7	-1.20 (0.2300)	60	36.1	579	21.0	-4.32 (<0.0001)
No	37	80.4	1,343	86.3		106	63.9	2,180	79.0	
Missing	0		7			0		13		
CWP										
Yes	8	17.4	95	6.1	-2.90 (0.0037)	43	25.9	216	7.8	-7.29 (<0.0001)
No	38	82.6	1,465	93.9		123	74.1	2,556	92.2	
Missing	0		3			0		0		

Table 2.3 (Continued): Characteristics of IBS cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square	Cases		Controls		Chi-Square
	No.	%	No.	%	statistic (p-value)*	No.	%	No.	%	statistic (p-value)*
CFS-like illness										
Yes	2	4.3	26	1.7	-1.34 (0.1799)	28	16.9	107	3.9	-6.95 (<0.0001)
No	44	95.7	1,537	98.3		138	83.1	2,665	96.1	
Missing	0		0			0		0		
BMI										
≤24.9 kg/m^2	11	23.9	522	33.4	2.6202 (0.6232)	69	41.6	1,381	49.8	4.8271 (0.3055)
25-29.9 kg/m^2	21	45.7	686	43.9		50	30.1	751	27.1	
≥30 kg/m^2	14	30.4	355	22.7		47	28.3	640	23.1	
Missing	0		0			0		0		
Mental Health Score										
< mean	19	43.2	390	25.6	-2.60 (0.0093)	81	49.7	905	33.5	-3.99 (<0.0001)
≥ mean	25	56.8	1,135	74.4		82	50.3	1,799	66.5	
Missing	2		38			3		68		
Mean (SE)	49.18 (1.31)		52.26 (0.22)		2.32 (0.0216)	46.40 (0.77)		50.29 (0.20)		4.92 (<0.0001)

Table 2.3 (Continued): Characteristics of IBS cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square	Cases		Controls		Chi-Square
	No.	%	No.	%	statistic (p-value)*	No.	%	No.	%	statistic (p-value)*
Physical Health Score										
< mean	20	45.5	336	22.0	-3.22 (0.0013)	87	53.4	691	25.6	-7.24 (<0.0001)
≥ mean	24	54.5	1,189	78.0		76	46.6	2,013	74.4	
Missing	2		38			3		68		
Mean (SE)	48.03 (1.26)		51.83 (0.23)		2.97 (0.0034)	44.98 (0.74)		51.32 (0.19)		8.41 (<0.0001)

Table 2.4: Association between IBS status and missing covariate information, AHP study, 1999.

	Case (212)		Control (4,336)		χ^2	p-value
	No.	%	No.	%		
Major Depressive Disorder						
Missing	0	0	20	0.46	0.9822	0.3217
Not missing	212	100	4316	99.54		
Chronic Widespread Pain						
Missing	0	0	3	0.07	0.1468	0.7016
Not missing	212	100	4333	99.93		
Gender						
Missing	0	0	1	0.02	0.0489	0.8250
Not missing	212	100	4335	99.98		
Physical self-rated health						
Missing	5	2.36	106	2.44	0.0063	0.9367
Not missing	207	97.64	4230	97.56		
Mental self-rated health						
Missing	5	2.36	106	2.44	0.0063	0.9367
Not missing	207	97.64	4230	97.56		
Chronic Fatigue Syndrome-like illness						
Missing	0	0	0	0	0	1.0
Not missing	212	100	4336	100		
Body Mass Index						
Missing	0	0	0	0	0	1.0
Not missing	212	100	4336	100		

Table 2.5: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, AHP study, 1999.

	Cases (203)		Controls (4,104)		OR	95% CI
	No.	%	No.	%		
Age at interview (years)						
< 36 years old	49	24.1	1,016	24.8	1.04	0.68, 1.60
36-43 years old	57	28.1	1,080	26.3	1.13	0.75, 1.71
44-51 years old	54	26.6	1,091	26.6	1.06	0.70, 1.61
> 51 years old	43	21.2	917	22.3	1.00	
Sex						
Male	44	21.7	1,478	36.0	1.00	
Female	159	78.3	2,626	64.0	2.03	1.44, 2.85
MDD						
Yes	64	31.5	762	18.6	2.00	1.48, 2.71
No	139	68.5	3,342	81.4	1.00	
CWP						
Yes	47	23.2	294	7.2	3.89	2.74, 5.53
No	156	76.8	3,810	92.8	1.00	
CFS-like illness						
Yes	29	14.3	129	3.1	5.13	3.30, 7.97
No	174	85.7	3,975	96.9	1.00	
BMI						
≤24.9 kg/m^2	75	36.9	1,744	42.5	1.00	
25-29.9 kg/m^2	70	34.5	1,393	33.9	1.17	0.83, 1.64
≥30 kg/m^2	58	28.6	967	23.6	1.40	0.98, 2.00

Table 2.5 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, AHP study, 1999.

	Cases (203)		Controls (4,104)		OR	95% CI
	No.	%	No.	%		
Mental Health Score						
< mean	98	48.3	1,266	30.8	2.09	1.56, 2.78
≥ mean	105	51.7	2,838	69.2	1.00	
Physical Health Score						
< mean	103	50.7	997	24.3	3.20	2.41, 4.26
≥ mean	100	49.3	3,107	75.7	1.00	
Continuous Covariates						
Age					1.00	0.98, 1.01
Mental Health Score					0.97	0.95, 0.98
Physical Health Score					0.95	0.94, 0.97

Table 2.6: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, by sex, AHP study, 1999.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age at interview (years)												
< 36 years old	8	18.2	295	20.0	0.83	0.34, 2.02	41	25.8	721	27.5	1.00	0.61, 1.65
36-43 years old	10	22.7	361	24.4	0.85	0.37, 1.95	47	29.6	719	27.4	1.13	0.69, 1.84
44-51 years old	13	29.5	423	28.6	0.94	0.43, 2.04	41	25.8	668	25.4	1.06	0.64, 1.75
> 51 years old	13	29.5	399	27.0	1.0		30	18.9	518	19.7	1.0	
MDD												
Yes	9	20.5	204	13.8	1.60	0.76, 3.38	55	34.6	558	21.2	1.93	1.38, 2.70
No	35	79.5	1,274	86.2	1.0		104	65.4	2,068	78.8	1.0	
CWP												
Yes	8	18.2	89	6.0	3.46	1.56, 7.68	39	24.5	205	7.8	3.81	2.57, 5.65
No	36	81.8	1,389	94.0	1.0		120	75.5	2,421	92.2	1.0	
CFS-like illness												
Yes	2	4.5	26	1.8	2.72	0.64, 11.52	27	17.0	103	3.9	5.01	3.14, 8.00
No	42	95.5	1,452	98.2	1.0		132	83.0	2,523	96.1	1.0	
BMI												
≤24.9 kg/m^2	11	25.0	466	31.5	1.0		64	40.3	1,278	48.9	1.0	
25-29.9 kg/m^2	21	47.7	668	45.2	1.34	0.64, 2.80	49	30.8	725	27.6	1.36	0.92, 2.00
≥30 kg/m^2	12	27.3	344	23.3	1.48	0.64, 3.39	46	28.9	623	23.7	1.48	1.00, 2.20

Table 2.6 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, by sex, AHP study, 1999.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Mental Health Score												
< mean	19	43.2	382	25.8	2.17	1.18, 3.99	79	49.7	884	33.7	1.94	1.39, 2.69
≥ mean	25	56.8	1,096	74.2	1.0		80	50.3	1,742	66.3	1.0	
Physical Health Score												
< mean	20	45.5	329	22.3	2.93	1.60, 5.36	83	52.2	668	25.4	3.20	2.31, 4.43
≥ mean	24	54.5	1,149	77.7	1.0		76	47.8	1,958	74.6	1.0	
Continuous Covariates												
Age					1.00	0.97, 1.03					1.00	0.98, 1.02
Mental Health Score					0.97	0.94, 1.00					0.97	0.95, 0.98
Physical Health Score					0.96	0.94, 0.99					0.95	0.94, 0.97

Table 2.7: Assessing effect measure modification for MDD-IBS model, AHP study, 1999.

	OR, (95% CI) for MDD	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		2.02 (1.49, 2.74)	4.3499 (0.2261)	Maybe
< 36 years old	2.43 (1.33, 4.43)			
36-43 years old	2.92 (1.68, 5.07)			
44-51 years old	1.34 (0.70, 2.54)			
> 51 years old	1.98 (1.17, 3.35)			
Sex		1.89 (1.39, 2.58)	0.2267 (0.6340)	No
Male	1.61 (0.76, 3.39)			
Female	1.96 (1.40, 2.75)			
CWP		1.75 (1.28, 2.38)	2.6681 (0.1024)	Yes
Yes	1.12 (0.59, 2.12)			
No	2.05 (1.44, 2.91)			
CFS-like illness		1.57 (1.15, 2.15)	15.0354 (0.0001)	Yes
Yes	0.39 (0.17, 0.89)			
No	2.11 (1.51, 2.95)			
BMI		2.00 (1.47, 2.72)	0.0617 (0.9696)	No
≤24.9 kg/m^2	2.01 (1.21, 3.34)			
25-29.9 kg/m^2	1.90 (1.11, 3.25)			
≥30 kg/m^2	2.10 (1.20, 3.66)			
Mental Health Score		1.66 (1.21, 2.28)	0.2849 (0.5935)	Maybe
< mean	1.55 (1.02, 2.35)			
≥ mean	1.85 (1.13, 3.02)			
Physical Health Score		1.77 (1.30, 2.41)	2.2758 (0.1314)	Yes
< mean	1.42 (0.91, 2.19)			
≥ mean	2.27 (1.47, 3.51)			

Table 2.8: Evaluation of confounding for the association between lifetime MDD and risk of lifetime IBS, AHP study, 1999.

	Lifetime MDD and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for lifetime MDD 2.00 (1.48, 2.71)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	3,481 (no lifetime MDD)		
Age (years)				
Continuous	0.99 (0.99, 1.00)	1.01 (0.99, 1.02)	2.00 (1.48, 2.70)	0
< 36 years old	1.22 (0.97, 1.53)	0.87 (0.53, 1.45)		
36-43 years old	1.20 (0.95, 1.50)	0.90 (0.55, 1.47)		
44-51 years old	1.16 (0.92, 1.46)	1.08 (0.67, 1.73)		
> 51 years old	1.0		2.00 (1.48, 2.70)	0
Sex				
Male	1.0			
Female	1.72 (1.45, 2.05)	1.83 (1.24, 2.70)	1.88 (1.39, 2.55)	0.0619
CWP				
Yes	2.30 (1.81, 2.92)	4.44 (2.89, 6.84)	1.76 (1.28, 2.41)	0.1278
No	1.0			
CFS-like illness				
Yes	5.76 (4.17, 7.97)	9.82 (5.56, 17.33)	1.63 (1.16, 2.30)	0.2045
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		1.98 (1.46, 2.69)	0.0101
25-29.9 kg/m^2	0.96 (0.80, 1.15)	1.19 (0.80, 1.78)		
≥30 kg/m^2	1.26 (1.04, 1.54)	1.34 (0.87, 2.05)		
Mental Health Score				
Continuous	0.93 (0.92, 0.94)	0.96 (0.95, 0.98)	1.53 (1.09, 2.15)	0.2679
< mean	3.69 (3.14, 4.33)	1.95 (1.37, 2.77)		
≥ mean	1.0		1.65 (1.20, 2.28)	0.1924
Physical Health Score				
Continuous	0.98 (0.97, 0.99)	0.95 (0.94, 0.96)	1.78 (1.31, 2.43)	0.1165
< mean	1.66 (1.41, 1.96)	3.52 (2.50, 4.97)		
≥ mean	1.0		1.77 (1.30, 2.41)	0.1222

Table 2.9: Assessing confounding and effect measure modification using backward elimination for the model of lifetime MDD, AHP study, 1999.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
Lifetime MDD	0.6946 (0.1545)	1.6820 (0.8170)	0.6329 (0.1545)
|ln(crude/adj)| of beta		0.88	0.98
Compare to:		Min Model	Full
-2log L	1617.9962	1599.684	1602.5418
df	1	5	3
Chi-square p-value			2.8578 (p>0.2)
OR (95% CI) for main effect:			
lifetime MDD	2.00 (1.48, 2.71)	5.38 (1.08, 26.66)	1.88 (1.39, 2.55)
Crude vs Adjusted			
|ln(crude/adj)|		0.99	1.05
Notes/Conclusions about current model:	MDL, adjusted for twin relatedness.	First-order interactions with age and sex, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.9513)

Table 2.9 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of lifetime MDD, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure: **Lifetime MDD**	0.6327 (0.1548)	0.6946 (0.1545)	0.6946 (0.1545)
|ln(crude/adj)| of beta	0.0003	0.09	0.09
Compare to:	Model 2	Model 3	Model 3
-2log L	1602.5494	1617.9962	1617.9962
df	2	1	1
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect: **lifetime MDD**	1.88 (1.39, 2.55)	2.0 (1.48, 2.71)	2.0 (1.48, 2.71)
Crude vs Adjusted |ln(crude/adj)|	0	0.06	0.06
Notes/Conclusions about current model:	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect did not change by >10%. Gender is not confounder.	OR for main effect is not confounded by any potential covariates examined, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	Gender (0.0002)	none	

Table 2.10: Assessing effect measure modification for gender-IBS model, AHP study, 1999.

	OR, (95% CI) for gender	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		2.05 (1.45, 2.88)	0.3448 (0.9514)	No
< 36 years old	2.09 (0.97, 4.53)			
36-43 years old	2.36 (1.18, 4.72)			
44-51 years old	2.00 (1.06, 3.77)			
> 51 years old	1.78 (0.92, 3.45)			
MDD		1.92 (1.36, 2.71)	0.2271 (0.6337)	No
Yes	2.23 (1.08, 4.60)			
No	1.83 (1.24, 2.70)			
CWP		1.95 (1.38, 2.74)	0.0504 (0.8224)	No
Yes	2.12 (0.95, 4.71)			
No	1.91 (1.31, 2.79)			
CFS-like illness		1.89 (1.34, 2.66)	0.06679 (0.4138)	Maybe
Yes	3.41 (0.76, 15.26)			
No	1.81 (1.27, 2.57)			
BMI		2.13 (1.51, 3.01)	0.0017 (0.9992)	No
≤24.9 kg/m^2	2.12 (1.11, 4.06)			
25-29.9 kg/m^2	2.15 (1.28, 3.62)			
≥30 kg/m^2	2.12 (1.11, 4.05)			
Mental Health Score		1.91 (1.36, 2.69)	0.1053 (0.7455)	No
< mean	1.80 (1.07, 3.01)			
≥ mean	2.01 (1.28, 3.17)			
Physical Health Score		1.94 (1.38, 2.73)	0.0739 (0.7858)	No
< mean	2.04 (1.23, 3.39)			
≥ mean	1.86 (1.17, 2.96)			

Table 2.11: Evaluation of confounding for the association between gender and risk of lifetime IBS, AHP study, 1999.

	Gender and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for gender 2.03 (1.44, 2.85)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	1,522 (male)		
Age (years)				
Continuous	0.97 (0.97, 0.98)	1.00 (0.97, 1.03)	2.03 (1.44, 2.86)	0
< 36 years old	1.92 (1.58, 2.34)	0.83 (0.34, 2.02)		
36-43 years old	1.56 (1.29, 1.89)	0.85 (0.37, 1.95)		
44-51 years old	1.22 (1.01, 1.46)	0.94 (0.43, 2.04)		
> 51 years old	1.0		2.03 (1.44, 2.86)	0
MDD				
Yes	1.56 (1.34, 1.83)	1.60 (0.76, 3.38)	1.91 (1.36, 2.69)	0.0609
No	1.0			
CWP				
Yes	1.30 (1.05, 1.61)	3.46 (1.56, 7.68)	1.94 (1.38, 2.74)	0.0453
No	1.0			
CFS-like illness				
Yes	2.18 (1.58, 3.01)	2.72 (0.64, 11.52)	1.88 (1.34, 2.65)	0.0768
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		2.12 (1.50, 3.00)	0.0434
25-29.9 kg/m^2	0.45 (0.39, 0.52)	1.34 (0.64, 2.80)		
≥30 kg/m^2	0.69 (0.58, 0.81)	1.48 (0.64, 3.39)		
Mental Health Score				
Continuous	0.98 (0.97, 0.99)	0.97 (0.94, 1.00)	1.88 (1.33, 2.64)	0.0768
< mean	1.39 (1.22, 1.58)	2.17 (1.18, 3.99)		
≥ mean	1.0		1.91 (1.35, 2.69)	0.0609
Physical Health Score				
Continuous	0.99 (0.99, 1.00)	0.96 (0.94, 0.99)	1.96 (1.39, 2.76)	0.0351
< mean	1.18 (1.03, 1.35)	2.93 (1.60, 5.36)		
≥ mean	1.0		1.94 (1.37, 2.73)	0.0453

Table 2.12: Assessing confounding and effect measure modification using backward elimination for the model of gender, AHP study, 1999.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**	**Model 3/FINAL MODEL β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:				
Gender	0.7061 (0.1740)	0.7474 (0.5024)	0.7523 (0.1769)	0.7061 (0.1740)
|ln(crude/adj)| of beta		0.06	0.01	0.06
Compare to:		Min Model	Full	Model 2
-2log L	1617.7616	1612.3442	1612.3442	1617.7616
df	1	3	2	1
Chi-square p-value			0 (p>0.2)	N/A
OR (95% CI) for main effect:				
Gender	2.03 (1.44, 2.85)	2.11 (0.79, 5.65)	2.12 (1.50, 3.00)	2.03 (1.44, 2.85)
Crude vs Adjusted |ln(crude/adj)|		0.04	0.005	0.04
Notes/Conclusions about current model:	gender, adjusted for twin relatedness.	First-order interaction with BMI and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.	OR for main effect is not confounded by any potential covariates, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.0699)	none

Table 2.13: Assessing effect measure modification for CWP-IBS model, AHP study, 1999.

	OR, (95% CI) for CWP	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		4.02 (2.83, 5.72)	11.7795 (0.0082)	Yes
< 36 years old	1.16 (0.27, 4.96)			
36-43 years old	9.19 (5.03, 16.80)			
44-51 years old	2.86 (1.46, 5.62)			
> 51 years old	3.58 (1.81, 7.09)			
MDD		3.47 (2.45, 4.93)	2.6944 (0.1007)	Yes
Yes	2.42 (1.34, 4.39)			
No	4.44 (2.89, 6.82)			
Gender		3.77 (2.66, 5.34)	0.0504 (0.8223)	No
Male	3.47 (1.57, 7.68)			
Female	3.84 (2.60, 5.66)			
CFS-like illness		3.02 (2.06, 4.42)	0.1327 (0.7157)	No
Yes	3.43 (1.47, 7.99)			
No	2.88 (1.89, 4.41)			
BMI		3.74 (2.64, 5.31)	5.5494 (0.0624)	Yes
≤24.9 kg/m^2	3.72 (1.93, 7.15)			
25-29.9 kg/m^2	6.06 (3.51, 10.44)			
≥30 kg/m^2	2.23 (1.17, 4.26)			
Mental Health Score		3.50 (2.46, 4.96)	0.7960 (0.3723)	Maybe
< mean	3.05 (1.88, 4.94)			
≥ mean	4.18 (2.53, 6.93)			
Physical Health Score		2.58 (1.78, 3.73)	0.0644 (0.7997)	No
< mean	2.52 (1.64, 3.87)			
≥ mean	2.80 (1.37, 5.71)			

Table 2.14: Evaluation of confounding for the association between CWP and risk of lifetime IBS, AHP study, 1999.

	CWP and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for CWP 3.89 (2.74, 5.53)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	3,966 (no CWP)		
Age (years)				
Continuous	1.05 (1.03, 1.06)	0.99 (0.97, 1.01)	4.06 (2.84, 5.81)	0.0428
< 36 years old	0.28 (0.19, 0.41)	1.32 (0.82, 2.12)		
36-43 years old	0.57 (0.42, 0.77)	0.99 (0.61, 1.63)		
44-51 years old	0.81 (0.61, 1.07)	1.16 (0.71, 1.89)		
> 51 years old	1.0		4.02 (2.81, 5.75)	0.0329
MDD				
Yes	2.35 (1.85, 2.98)	2.04 (1.44, 2.90)	3.54 (2.47, 5.09)	0.0943
No	1.0			
Gender				
Male	1.0			
Female	1.41 (1.10, 1.79)	1.91 (1.30, 2.79)	3.76 (2.64, 5.34)	0.0340
CFS-like illness				
Yes	9.74 (6.93, 13.70)	3.09 (1.56, 6.09)	2.98 (2.05, 4.33)	0.2665
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		3.78 (2.64, 5.42)	0.0287
25-29.9 kg/m^2	1.59 (1.20, 2.10)	0.97 (0.66, 1.43)		
≥30 kg/m^2	2.44 (1.85, 3.22)	1.38 (0.93, 2.04)		
Mental Health Score				
Continuous	0.97 (0.96, 0.98)	0.96 (0.95, 0.98)	3.49 (2.43, 5.01)	0.1085
< mean	2.02 (1.61, 2.53)	2.02 (1.46, 2.80)		
≥ mean	1.0		3.52 (2.46, 5.03)	0.0999
Physical Health Score				
Continuous	0.91 (0.90, 0.92)	0.96 (0.95, 0.98)	2.46 (1.63, 3.72)	0.4582
< mean	6.94 (5.48, 8.79)	2.64 (1.90, 3.67)		
≥ mean	1.0		2.58 (1.77, 3.76)	0.4106

Table 2.15: Assessing confounding and effect measure modification using backward elimination for the model of CWP, AHP study, 1999.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**	**Model 3 β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:				
CWP	1.3585 (0.1797)	0.4415 (1.1276)	1.3191 (0.1862)	1.2847 (0.1836)
|ln(crude/adj)| of beta		1.12	1.09	0.03
Compare to:		Min Model	Full	Model 2
-2log L	1588.792	1567.158	1568.7278	1570.1196
df	1	7	4	3
Chi-square p-value			1.5698 (p>0.2)	N/A
OR (95% CI) for main effect:				
CWP	3.89 (2.74, 5.53)	1.56 (0.17, 14.18)	3.74 (2.60, 5.39)	3.61 (2.52, 5.18)
Crude vs Adjusted |ln(crude/adj)|		0.91	0.87	0.04
Notes/Conclusions about current model:	CWP, adjusted for twin relatedness.	First-order interactions with gender, age, and BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.	OR for main effect did not change by >10%. Age is not confounder.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.2221)	BMI (0.2994)

Table 2.15 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of CWP, AHP study, 1999.

	Model 4 **β (SE)**	**Model 5** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure: **CWP**	1.3232 (0.1800)	1.3585 (0.1797)	1.3585 (0.1797)
|ln(crude/adj)| of beta	0.03	0.03	0.03
Compare to:	Model 3	Model 4	Model 4
-2log L	1572.5696	1588.792	1588.792
df	2	1	1
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect: **CWP**	3.76 (2.64, 5.34)	3.89 (2.74, 5.53)	3.89 (2.74, 5.53)
Crude vs Adjusted |ln(crude/adj)|	0.04	0.03	0.03
Notes/Conclusions about current model:	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect did not change by >10%. Gender is not confounder.	OR for main effect is not confounded by any potential confounders, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	Gender (0.0001)	none	

Table 2.16: Assessing effect measure modification for CFS-like illness and IBS model, AHP study, 1999.

	OR, (95% CI) for CF	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		5.08 (3.31, 7.81)	12.11 (0.007)	Yes
< 36 years old	2.57 (0.87, 7.55)			
36-43 years old	13.74 (6.68, 28.28)			
44-51 years old	2.32 (0.88, 6.09)			
> 51 years old	6.03 (2.33, 15.65)			
MDD		3.84 (2.47, 5.95)	15.96 (<0.0001)	Yes
Yes	1.79 (0.90, 3.58)			
No	9.79 (5.55, 17.29)			
Gender		4.71 (3.05, 7.27)	0.67 (0.415)	Maybe
Male	2.66 (0.61, 11.57)			
Female	5.01 (3.17, 7.92)			
CWP		3.40 (2.12, 5.46)	0.13 (0.717)	No
Yes	3.66 (1.89, 7.09)			
No	3.08 (1.56, 6.05)			
BMI		5.08 (3.30, 7.83)	0.49 (0.785)	No
≤24.9 kg/m^2	4.05 (1.84, 8.87)			
25-29.9 kg/m^2	5.58 (2.75, 11.35)			
≥30 kg/m^2	5.72 (2.67, 12.25)			
Mental Health Score		4.38 (2.82, 6.81)	0.098 (0.754)	No
< mean	4.59 (2.68, 7.87)			
≥ mean	3.95 (1.84, 8.51)			
Physical Health Score		3.84 (2.46, 6.01)	0.43 (0.510)	Maybe
< mean	4.19 (2.47, 7.11)			
≥ mean	2.99 (1.26, 7.07)			

Table 2.17: Evaluation of confounding for the association between CFS-like illness and risk of lifetime IBS, AHP study, 1999.

	CFS-like illness and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for CFS-like illness 5.13 (3.30, 7.97)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	4,149 (no CF)		
Age (years)				
Continuous	1.00 (0.98, 1.02)	1.00 (0.98, 1.01)	5.13 (3.30, 7.97)	0
< 36 years old	1.14 (0.70, 1.86)	1.11 (0.71, 1.74)		
36-43 years old	1.09 (0.67, 1.78)	0.98 (0.63, 1.54)		
44-51 years old	1.45 (0.92, 2.30)	1.13 (0.73, 1.76)		
> 51 years old	1.0		5.13 (3.30, 7.99)	0
MDD				
Yes	6.11 (4.42, 8.45)	2.10 (1.51, 2.93)	4.23 (2.58, 6.93)	0.1929
No	1.0			
Gender				
Male	1.0			
Female	2.60 (1.72, 3.94)	1.81 (1.27, 2.57)	4.67 (3.01, 7.26)	0.0939
CWP				
Yes	9.74 (6.92, 13.70)	2.87 (1.87, 4.40)	3.36 (2.10, 5.36)	0.4232
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		5.06 (3.26, 7.85)	0.0137
25-29.9 kg/m^2	1.22 (0.84, 1.78)	1.11 (0.77, 1.59)		
≥30 kg/m^2	1.36 (0.90, 2.04)	1.30 (0.89, 1.90)		
Mental Health Score				
Continuous	0.95 (0.94, 0.96)	0.97 (0.96, 0.98)	4.14 (2.61, 6.56)	0.2144
< mean	3.13 (2.26, 4.33)	1.85 (1.36, 2.51)		
≥ mean	1.0		4.36 (2.79, 6.81)	0.1626
Physical Health Score				
Continuous	0.94 (0.93, 0.95)	0.96 (0.95, 0.97)	3.57 (2.21, 5.76)	0.3625
< mean	3.76 (2.73, 5.18)	2.78 (2.04, 3.78)		
≥ mean	1.0		3.79 (2.42, 5.94)	0.3027

Table 2.18: Assessing confounding and effect measure modification using backward elimination for the model of CFS-like illness, AHP study, 1999.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
CFS-like illness	1.6345 (0.2250)	1.0631 (1.7462)	1.5144 (0.2247)
|ln(crude/adj)| of beta		0.43	0.35
Compare to:		Min Model	Full
-2log L	1594.9494	1575.6346	1576.2848
df	1	7	4
Chi-square p-value			0.6502 (p>0.2)
OR (95% CI) for main effect:			
CFS-like illness	5.13 (3.30, 7.97)	2.90 (0.09, 88.73)	4.55 (2.93, 7.06)
Crude vs Adjusted |ln(crude/adj)|		0.57	0.45
Notes/Conclusions about current model:	CFS-like illness, adjusted for twin relatedness.	First-order interactions with gender, BMI, and age, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.7285)

Table 2.18 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of CFS-like illness, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**Model 5** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:				
CFS-like illness	1.5178 (0.2247)	1.5419 (0.2246)	1.6345 (0.2250)	1.5419 (0.2246)
|ln(crude/adj)| of beta	0.002	0.02	0.06	0.02
Compare to:	Model 2	Model 3	Model 4	Model 3
-2log L	1576.6758	1580.4936	1594.9494	1580.4936
df	3	2	1	2
Chi-square p-value	N/A	N/A	N/A	N/A
OR (95% CI) for main effect:				
CFS-like illness	4.56 (2.94, 7.09)	4.67 (3.01, 7.26)	5.13 (3.30, 7.97)	4.67 (3.01, 7.26)
Crude vs Adjusted				
|ln(crude/adj)|	0.002	0.02	0.094	0.02
Notes/Conclusions about current model:	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect did change by >10%. Gender is confounder, return it to model.	OR for main effect is confounded by gender, and adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	BMI (0.0471)	Gender (0.0003)	None	

Table 2.19: Assessing effect measure modification for mental self-rated health and IBS model, AHP study, 1999.

	OR, (95% CI) for Mental self-rated health	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		2.10 (1.58, 2.79)	2.9393 (0.4011)	Maybe
< 36 years old	1.66 (0.94, 2.95)			
36-43 years old	3.11 (1.80, 5.36)			
44-51 years old	1.87 (1.07, 3.26)			
> 51 years old	1.89 (1.01, 3.54)			
MDD		1.84 (1.37, 2.47)	0.2850 (0.5935)	Maybe
Yes	1.63 (0.95, 2.80)			
No	1.94 (1.37, 2.76)			
Gender		1.99 (1.50, 2.65)	0.1054 (0.7454)	No
Male	2.18 (1.19, 4.00)			
Female	1.95 (1.41, 2.68)			
CWP		1.89 (1.42, 2.51)	0.7903 (0.3740)	Maybe
Yes	1.48 (0.80, 2.75)			
No	2.03 (1.47, 2.80)			
CFS-like illness		1.88 (1.41, 2.51)	0.0981 (0.7542)	Maybe
Yes	2.14 (0.88, 5.20)			
No	1.85 (1.36, 2.51)			
BMI		2.08 (1.56, 2.76)	1.8496 (0.3966)	Maybe
≤24.9 kg/m^2	1.61 (1.00, 2.57)			
25-29.9 kg/m^2	2.46 (1.52, 3.98)			
≥30 kg/m^2	2.36 (1.39, 4.03)			
Physical Health Score		1.94 (1.46, 2.59)	0.0304 (0.8616)	No
< mean	1.99 (1.33, 2.99)			
≥ mean	1.89 (1.27, 2.83)			

Table 2.20: Evaluation of confounding for the association between mental self-rated health and risk of lifetime IBS, AHP study, 1999.

	Mental self-rated health and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for Mental health score 2.09 (1.56, 2.78)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	2,943 (MH >50)		
Age (years)				
Continuous	0.98 (0.97, 0.99)	1.00 (0.98, 1.02)	2.08 (1.56, 2.78)	0.0048
< 36 years old	1.63 (1.34, 1.97)	1.03 (0.58, 1.83)		
36-43 years old	1.40 (1.15, 1.69)	0.83 (0.46, 1.49)		
44-51 years old	1.15 (0.94, 1.39)	1.06 (0.61, 1.83)		
> 51 years old	1.0		2.09 (1.57, 2.79)	0
MDD				
Yes	3.68 (3.14, 4.32)	1.84 (1.13, 2.99)	1.85 (1.37, 2.50)	0.1220
No	1.0			
Gender				
Male	1.0			
Female	1.47 (1.28, 1.69)	2.00 (1.27, 3.15)	1.99 (1.49, 2.66)	0.0490
CWP				
Yes	2.00 (1.60, 2.50)	4.12 (2.48, 6.84)	1.89 (1.41, 2.54)	0.1006
No	1.0			
CFS-like illness				
Yes	2.97 (2.15, 4.09)	3.90 (1.84, 8.29)	1.88 (1.40, 2.52)	0.1059
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		2.07 (1.55, 2.76)	0.0096
25-29.9 kg/m^2	0.96 (0.83, 1.12)	0.98 (0.62, 1.56)		
≥30 kg/m^2	1.22 (1.04, 1.44)	1.15 (0.70, 1.88)		
Physical Health Score				
Continuous	1.00 (0.99, 1.01)	0.96 (0.94, 0.97)	2.04 (1.52, 2.73)	0.0242
< mean	1.39 (1.20, 1.61)	3.00 (2.01, 4.47)		
≥ mean	1.0		1.93 (1.44, 2.59)	0.0796

Table 2.21: Assessing confounding and effect measure modification using backward elimination for the model of mental self-rated health, AHP study, 1999.

	Min Model β **(SE)**	**Full Model** β **(SE)**	**Model 2** β **(SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
Mental Health Score	-0.0352 (0.0059)	-0.0760 (0.0323)	-0.0285 (0.0065)
|ln(crude/adj)| of beta		0.77	0.98
Compare to:		Min Model	Full
-2log L	1606.2196	1593.3566	1597.4954
df	1	7	4
Chi-square p-value			4.1388 (p>0.2)
OR (95% CI) for main effect:			
Mental Health Score	0.97 (0.95, 0.98)	0.93 (0.87, 0.99)	0.97 (0.96, 0.98)
Crude vs Adjusted |ln(crude/adj)|		0.04	0.04
Notes/Conclusions about current model:	Mental self-rated health, adjusted for twin relatedness.	First-order interactions with BMI, age, and MDL, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.7585)

Table 2.21 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of mental self-rated health, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**Model 5** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure: **Mental Health Score**	-0.0286 (0.0065)	-0.0289 (0.0065)	-0.0352 (0.0059)	-0.0352 (0.0059)
|ln(crude/adj)| of beta	0.004	0.01	0.20	0.20
Compare to:	Model 2	Model 3	Model 4	Model 4
-2log L	1597.5442	1600.0432	1606.2196	1606.2196
df	3	2	1	1
Chi-square p-value	N/A	N/A	N/A	N/A
OR (95% CI) for main effect: **Mental Health Score**	0.97 (0.96, 0.98)	0.97 (0.96, 0.98)	0.97 (0.95, 0.98)	0.97 (0.95, 0.98)
Crude vs Adjusted				
|ln(crude/adj)|	0	0.01	0	0
Notes/Conclusions about current model:	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect did not change by >10%. MDL is not confounder.	OR for main effect not confounded by any covariates, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	BMI (0.2822)	MDL (0.0144)	none	

Table 2.22: Assessing effect measure modification for physical self-rated health and IBS model, AHP study, 1999.

	OR, (95% CI) for Physical self-rated health	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		3.38 (2.53, 4.53)	10.3898 (0.0155)	Yes
< 36 years old	2.82 (1.51, 5.25)			
36-43 years old	6.30 (3.61, 11.00)			
44-51 years old	1.81 (1.03, 3.18)			
> 51 years old	4.05 (2.08, 7.87)			
MDD		3.01 (2.26, 4.00)	2.2726 (0.1317)	Yes
Yes	2.19 (1.31, 3.66)			
No	3.52 (2.50, 4.95)			
Gender		3.14 (2.36, 4.17)	0.0739 (0.7857)	No
Male	2.91 (1.59, 5.33)			
Female	3.20 (2.32, 4.42)			
CWP		2.59 (1.91, 3.52)	0.0647 (0.7991)	No
Yes	2.38 (1.11, 5.11)			
No	2.65 (1.91, 3.68)			
CFS-like illness		2.89 (2.16, 3.87)	0.4376 (0.5083)	Maybe
Yes	3.89 (1.49, 10.19)			
No	2.77 (2.04, 3.77)			
BMI		3.17 (2.36, 4.25)	0.6036 (0.7395)	Maybe
≤24.9 kg/m^2	3.72 (2.31, 5.99)			
25-29.9 kg/m^2	3.01 (1.85, 4.88)			
≥30 kg/m^2	2.86 (1.64, 4.99)			
Mental Health Score		3.07 (2.31, 4.08)	0.0304 (0.8615)	No
< mean	3.15 (2.08, 4.78)			
≥ mean	2.99 (2.02, 4.43)			

Table 2.23: Evaluation of confounding for the association between physical self-rated health and risk of lifetime IBS, AHP study, 1999.

	Physical self-rated health and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for Physical health score 3.20 (2.41, 4.26)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	3,207 (PH >50)		
Age (years)				
Continuous	1.05 (1.04, 1.05)	0.99 (0.97, 1.01)	3.42 (2.54, 4.61)	-0.0665
< 36 years old	0.30 (0.25, 0.38)	1.73 (0.88, 3.42)		
36-43 years old	0.51 (0.42, 0.62)	1.12 (0.54, 2.31)		
44-51 years old	0.60 (0.50, 0.73)	1.84 (0.93, 3.63)		
> 51 years old	1.0		3.37 (2.52, 4.52)	0.0518
MDD				
Yes	1.63 (1.38, 1.92)	2.26 (1.46, 3.50)	3.05 (2.28, 4.07)	0.0480
No	1.0			
Gender				
Male	1.0			
Female	1.23 (1.06, 1.42)	1.85 (1.16, 2.94)	3.13 (2.35, 4.17)	0.0221
CWP				
Yes	6.49 (5.16, 8.17)	2.76 (1.35, 5.64)	2.61 (1.92, 3.53)	0.2038
No	1.0			
CFS-like illness				
Yes	3.42 (2.49, 4.69)	3.01 (1.28, 7.09)	2.87 (2.15, 3.84)	0.1088
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		3.20 (2.37, 4.30)	0
25-29.9 kg/m^2	1.64 (1.39, 1.94)	1.13 (0.72, 1.78)		
≥30 kg/m^2	3.43 (2.88, 4.10)	1.15 (0.67, 1.96)		
Mental Health Score				
Continuous	0.99 (0.98, 1.00)	0.97 (0.95, 0.98)	3.09 (2.32, 4.12)	0.0350
< mean	1.38 (1.20, 1.60)	1.88 (1.25, 2.83)		
≥ mean	1.0		3.06 (2.30, 4.08)	0.0447

Table 2.24: Assessing confounding and effect measure modification using backward elimination for the model of physical self-rated health, AHP study, 1999.

	Min Model β **(SE)**	**Full Model** β **(SE)**	**Model 2** β **(SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
Physical Health Score	-0.0464 (0.0058)	-0.0905 (0.0350)	-0.0499 (0.0063)
|ln(crude/adj)| of beta		0.67	0.60
Compare to:		Min Model	Full
-2log L	1581.9058	1573.7552	1576.3028
df	1	5	3
Chi-square p-value			2.5476 (p>0.2)
OR (95% CI) for main effect:			
Physical Health Score	0.95 (0.94, 0.97)	0.91 (0.85, 0.98)	0.95 (0.94, 0.96)
Crude vs Adjusted |ln(crude/adj)|		0.04	0.04
Notes/Conclusions about current model:	STDPH, adjusted for twin relatedness.	First-order interactions with age and BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.8419)

Table 2.24 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of physical self-rated health, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure: **Physical Health Score**	-0.0504 (0.0062)	-0.0464 (0.0058)	-0.0464 (0.0058)
|ln(crude/adj)| of beta	0.01	0.08	0.08
Compare to:	Model 2	Model 3	Model 3
-2log L	1576.6368	1581.9058	1581.9058
df	2	1	1
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect: **Physical Health Score**	0.95 (0.94, 0.96)	0.95 (0.94, 0.97)	0.95 (0.94, 0.97)
Crude vs Adjusted |ln(crude/adj)|	0	0	0
Notes/Conclusions about current model:	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect is not confounded by any covariates, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	Age (0.0178)	none	

Table 2.25: Assessing effect measure modification for age-IBS model, AHP study, 1999.

	OR, (95% CI) for age	$OR_{M\text{-}H}$ (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
MDD		0.98 (0.74, 1.30)	5.0467 (0.0247)	Yes
Yes	1.61 (0.96, 2.71)			
No	0.79 (0.56, 1.11)			
Gender		0.94 (0.71, 1.25)	0.0247 (0.8750)	No
Male	0.90 (0.49, 1.66)			
Female	0.95 (0.69, 1.31)			
CWP		1.12 (0.84, 1.49)	0.6057 (0.4364)	Maybe
Yes	1.41 (0.74, 1.66)			
No	1.06 (0.77, 1.46)			
CFS-like illness		1.00 (0.75, 1.33)	2.2476 (0.1338)	Yes
Yes	1.79 (0.79, 4.05)			
No	0.92 (0.68, 1.25)			
BMI		1.03 (0.77, 1.36)	0.2119 (0.8995)	No
≤24.9 kg/m^2	1.00 (0.63, 1.59)			
25-29.9 kg/m^2	0.97 (0.60, 1.59)			
≥30 kg/m^2	1.14 (0.67, 1.94)			
Physical Health Score				
< mean	1.43 (0.95, 2.16)	1.19 (0.89, 1.58)	1.5119 (0.2188)	Maybe
≥ mean	1.00 (0.67, 1.49)			
Mental Health Score		0.93 (0.70, 1.24)	0.0250 (0.8744)	No
< mean	0.91 (0.60, 1.38)			
≥ mean	0.95 (0.64, 1.41)			

Table 2.26: Evaluation of confounding for the association between age and risk of lifetime IBS, AHP study, 1999.

	Age and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for age 1.00 (0.75, 1.33)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	Ln(OR_{crude} /OR_{adj})
Total=4,307	4,104 (controls)	2,246 (age ≥42.89)		
MDD				
Yes	1.00 (1.00, 1.01)	1.35 (0.85, 2.13)	0.99 (0.74, 1.31)	0.0101
No	1.0			
Gender				
Male	1.0			
Female	1.01 (0.95, 1.08)	1.98 (1.26, 3.11)	0.94 (0.71, 1.25)	0.0619
CWP				
Yes	0.97 (0.96, 0.98)	3.59 (2.28, 5.66)	1.13 (0.84, 1.51)	0.1222
No	1.0			
CFS-like illness				
Yes	1.00 (0.99, 1.01)	3.66 (1.92, 6.97)	1.01 (0.76, 1.34)	0.0099
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		1.03 (0.77, 1.38)	0.0296
25-29.9 kg/m^2	0.97 (0.91, 1.02)	1.19 (0.73, 1.94)		
≥30 kg/m^2	0.99 (0.96, 1.00)	1.33 (0.80, 2.22)		
Physical Health Score				
Continuous	1.00 (1.00, 1.00)	0.96 (0.95, 0.97)	1.24 (0.92, 1.68)	0.2151
< mean	0.97 (0.96, 0.98)	2.81 (1.88, 4.20)		
≥ mean	1.0	1.0	1.20 (0.89, 1.61)	0.1823
Mental Health Score				
Continuous	1.00 (1.00, 1.00)	0.97 (0.95, 0.98)	0.94 (0.71, 1.26)	0.0619
< mean	0.97 (0.92, 1.02)	2.11 (1.40, 3.18)		
≥ mean	1.0		0.94 (0.71, 1.25)	0.0619

Table 2.27: Assessing confounding and effect measure modification using backward elimination for the model of age, AHP study, 1999.

	Min Model β **(SE)**	**Full Model** β **(SE)**	**Model 2** β **(SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
age	-0.0037 (0.0073)	-0.0075 (0.0248)	-0.0030 (0.0075)
|ln(crude/adj)| of beta		0.71	0.92
Compare to:		Min Model	Full
-2log L	1636.3244	1612.1446	1612.2208
df	1	5	3
Chi-square p-value			0.0762 (p>0.2)
OR (95% CI) for main effect:			
age	1.00 (0.98, 1.01)	0.99 (0.95, 1.04)	1.00 (0.98, 1.01)
Crude vs Adjusted |ln(crude/adj)|		0.01	0.01
Notes/Conclusions about current model:	Age, adjusted for twin relatedness.	First-order interactions with gender and BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.0666)

Table 2.27 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of age, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
age	0.0000 (0.0074)	-0.0037 (0.0073)	-0.0037 (0.0073)
|ln(crude/adj)| of beta	0	0	0
Compare to:	Model 2	Model 3	Model 3
-2log L	1617.7604	1636.3244	1636.3244
df	2	1	1
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect:			
age	1.00 (0.99, 1.01)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)
Crude vs Adjusted |ln(crude/adj)|	0	0	0
Notes/Conclusions about current model:	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect did not change by >10%. Gender is not confounder.	OR for main effect is not confounded by any covariates, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	Gender (<0.0001)	None	

Table 2.28: Assessing effect measure modification for BMI-IBS model, AHP study, 1999.

	OR, (95% CI) for BMI	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		1.27 (0.95, 1.71)	0.2334 (0.9720)	No
< 36 years old	1.28 (0.72, 2.28)			
36-43 years old	1.35 (0.78, 2.34)			
44-51 years old	1.32 (0.74, 2.35)			
> 51 years old	1.10 (0.56, 2.18)			
MDD		1.25 (0.93, 1.68)	0 (0.9963)	No
Yes	1.25 (0.74, 2.12)			
No	1.25 (0.88, 1.78)			
Gender		1.40 (1.04, 1.88)	0.0023 (0.9620)	No
Male	1.38 (0.69, 2.76)			
Female	1.41 (1.02, 1.95)			
CWP		1.15 (0.85, 1.54)	0.0108 (0.9173)	No
Yes	1.19 (0.59, 2.39)			
No	1.14 (0.82, 1.58)			
CFS-like illness		1.23 (0.92, 1.65)	0.4948 (0.4818)	Maybe
Yes	1.66 (0.68, 4.04)			
No	1.19 (0.87, 1.62)			
Physical Health Score		1.02 (0.76, 1.38)	0.5993 (0.4389)	Maybe
< mean	0.90 (0.58, 1.40)			
≥ mean	1.14 (0.76, 1.70)			
Mental Health Score		1.25 (0.93, 1.68)	1.9092 (0.1670)	Yes
< mean	1.57 (1.01, 2.43)			
≥ mean	1.03 (0.70, 1.53)			

Table 2.29: Evaluation of confounding for the association between BMI and risk of lifetime IBS, AHP study, 1999.

	BMI and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for BMI 1.26 (0.94, 1.70)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=4,307	4,104 (controls)	1,819 (BMI<25)		
Age (years)				
Continuous	1.04 (1.03, 1.05)	0.99 (0.97, 1.02)	1.29 (0.95, 1.75)	0.0235
< 36 years old	0.34 (0.28, 0.42)	1.00 (0.48, 2.10)		
36-43 years old	0.55 (0.46, 0.67)	1.01 (0.48, 2.14)		
44-51 years old	0.66 (0.55, 0.80)	0.97 (0.44, 2.14)		
> 51 years old	1.0		1.28 (0.95, 1.73)	0.0157
MDD				
Yes	1.07 (0.92, 1.25)	2.00 (1.22, 3.30)	1.25 (0.93, 1.69)	0.0080
No	1.0			
Gender				
Male	1.0			
Female	0.50 (0.43, 0.57)	2.08 (1.09, 3.99)	1.40 (1.04, 1.89)	0.1054
CWP				
Yes	1.84 (1.45, 2.33)	3.73 (1.97, 7.09)	1.15 (0.85, 1.55)	0.0913
No	1.0			
CFS-like illness				
Yes	1.26 (0.92, 1.72)	4.10 (1.89, 8.91)	1.23 (0.92, 1.66)	0.0241
No	1.0			
Physical Health Score				
Continuous	0.96 (0.95, 0.97)	0.95 (0.93, 0.97)	1.04 (0.76, 1.41)	0.1919
< mean	2.20 (1.91, 2.55)	3.67 (2.29, 5.91)		
≥ mean	1.0		1.03 (0.76, 1.40)	0.2607
Mental Health Score				
Continuous	1.00 (0.99, 1.01)	0.98 (0.96, 1.00)	1.26 (0.93, 1.69)	0
< mean	1.06 (0.93, 1.21)	1.57 (0.97, 2.55)		
≥ mean	1.0		1.26 (0.93, 1.69)	0

Table 2.30: Assessing confounding and effect measure modification using backward elimination for the model of BMI, AHP study, 1999.

	Min Model β **(SE)**	**Full Model** β **(SE)**	**Model 2** β **(SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
BMI			
≤24.9 kg/m^2	0 (0)	0 (0)	0 (0)
25-29.9 kg/m^2	0.1567 (0.1729)	0.4219 (0.4751)	0.3043 (0.1777)
≥30 kg/m^2	0.3376 (0.1801)	0.6424 (0.9209)	0.4005 (0.1834)
|ln(crude/adj)| of beta		0.99; 0.64	0.33; 0.47
Compare to:		Min Model	Full
-2log L	1633.1152	1612.153	1612.2208
df	1	5	3
Chi-square p-value			0.0678 (p>0.2)
OR (95% CI) for main effect:			
BMI			
≤24.9 kg/m^2	1.00	1.00	1.00
25-29.9 kg/m^2	1.17 (0.83, 1.64)	1.52 (0.60, 3.87)	1.36 (0.96, 1.92)
≥30 kg/m^2	1.40 (0.98, 2.00)	1.90 (0.31, 11.56)	1.49 (1.04, 2.14)
Crude vs Adjusted		0.26	0.11
|ln(crude/adj)|		0.31	0.24
Notes/Conclusions about current model:	BMI, adjusted for twin relatedness.	First-order interactions with gender and age, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.6934)

Table 2.30 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of BMI, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (203/4,104)	4,307 (203/4,104)	4,307 (203/4,104)
Main Exposure:			
BMI			
≤24.9 kg/m^2	0 (0)	0 (0)	0 (0)
25-29.9 kg/m^2	0.2945 (0.1760)	0.1567 (0.1792)	0.2945 (0.1760)
≥30 kg/m^2	0.3910 (0.1807)	0.3376 (0.1801)	0.3910 (0.1807)
|ln(crude/adj)| of beta	0.03; 0.02	0.63; 0.15	0.03; 0.02
Compare to:	Model 2	Model 3	Model 2
-2log L	1612.3442	1633.1152	1612.3442
df	2	1	2
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect:			
BMI			
≤24.9 kg/m^2	1.00	1.00	1.00
25-29.9 kg/m^2	1.34 (0.95, 1.90)	1.17 (0.83, 1.64)	1.34 (0.95, 1.90)
≥30 kg/m^2	1.48 (1.04, 2.11)	1.40 (0.98, 2.00)	1.48 (1.04, 2.11)
Crude vs Adjusted	0.01	0.14	0.01
|ln(crude/adj)|	0.01	0.06	0.01
Notes/Conclusions about current model:	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect did change by >10%. Gender is confounder, return it to model.	OR for main effect is confounded by gender, and adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	Gender (<0.0001)	None	

Table 2.31: Characteristics of MDD cases and controls, AHP study, 1999 (Sensitivity analysis of timing).

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
Total	4,558		866	19.0	3,692	81.0	
Age at interview (years)							18.8083 (0.0159)
< 36 years old	1,121	24.6	223	25.8	898	24.3	
36-43 years old	1,201	26.4	242	28.0	959	26.0	
44-51 years old	1,208	26.5	231	26.7	977	26.5	
> 51 years old	1,026	22.5	169	19.5	857	23.2	
Missing	2		1		1		
Mean (SE)			42.76 (0.18)		42.84 (0.16)		0.63 (0.5296)
Sex							-5.71 (<0.0001)
Male	2,530	55.5	223	25.8	2,307	62.5	
Female	2,027	44.5	643	74.2	1,384	37.5	
Missing	1		0		1		
IBS							
Yes	212	4.7	69	8.0	143	3.9	-5.05 (<0.0001)
No	4,316	95.3	792	92.0	3,524	96.1	
Missing	30		5		25		
CWP							
Yes	363	8.0	123	14.2	240	6.5	-7.36 (<0.0001)
No	4,187	92.0	742	85.8	3,445	93.5	
Missing	8		1		7		
CFS-like illness							
Yes	162	3.6	94	10.9	68	1.8	-11.40 (<0.0001)
No	4,396	96.4	772	89.1	3,624	98.2	
Missing	0		0		0		

Table 2.31 (Continued): Characteristics of MDD cases and controls, AHP study, 1999.

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
BMI							
≤24.9 kg/m^2	1,986	43.6	367	42.4	1,619	43.9	9.3646 (0.0526)
25-29.9 kg/m^2	1,509	33.1	268	30.9	1,241	33.6	
≥30 kg/m^2	1,063	23.3	231	26.7	832	22.5	
Missing	0		0		0		
Mental Health Score							
< mean	1,397	31.5	480	56.4	917	25.6	-16.41 (<0.0001)
≥ mean	3,041	68.5	371	43.6	2,670	74.4	
Missing	120		15		105		
Mean (SE)			44.24 (0.31)		52.38 (0.15)		23.62 (<0.0001)
Physical Health Score							
< mean	1,137	25.6	291	34.2	846	23.6	-5.83 (<0.0001)
≥ mean	3,301	74.4	560	65.8	2,741	76.4	
Missing	120		15		105		
Mean (SE)			49.79 (0.32)		51.56 (0.16)		5.03 (<0.0001)

Table 2.32: Characteristics of MDD cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square statistic (p-value)*	Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%		No.	%	No.	%	
Total	223	13.9	1,384	86.1		643	21.8	2,307	78.2	
Age at interview (years)					5.6979 (0.4579)					17.7716 (0.0230)
< 36 years old	38	17.0	280	20.2		185	28.8	618	26.8	
36-43 years old	62	27.8	328	23.7		180	28.0	631	27.4	
44-51 years old	68	30.5	390	28.2		163	25.4	587	25.4	
> 51 years old	55	24.7	386	27.9		114	17.8	471	20.4	
Missing	0		0			1		0		
Mean (SE)	44.27 (0.26)		44.36 (0.25)		1.50 (0.1357)	41.97 (0.19)		41.98 (0.19)		0.33 (0.7382)
IBS										
Yes	9	4.1	37	2.7	-1.12 (0.2625)	60	9.4	106	4.6	-4.47 (<0.0001)
No	213	95.9	1,343	97.3		579	90.6	2,180	95.4	
Missing	1		4			4		21		
CWP										
Yes	25	11.3	78	5.7	-3.12 (0.0018)	98	15.2	162	7.0	-6.30 (<0.0001)
No	197	88.7	1,302	94.3		545	84.8	2,142	93.0	
Missing	1		4			0		3		

Table 2.32 (Continued): Characteristics of MDD cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square statistic (p-value)*	Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%		No.	%	No.	%	
CFS-like illness										
Yes	16	7.2	12	0.9	-5.61 (<0.0001)	78	12.1	56	2.4	-9.41 (<0.0001)
No	207	92.8	1,372	99.1		565	87.9	2,251	97.6	
Missing	0		0			0		0		
BMI										
≤24.9 kg/m^2	69	30.9	463	33.4	4.4929 (0.3434)	298	46.3	1,156	50.1	10.3189 (0.0354)
25-29.9 kg/m^2	104	46.6	600	43.4		164	25.5	640	27.7	
≥30 kg/m^2	50	22.4	321	23.2		181	28.1	511	22.1	
Missing	0		0			0		0		
Mental Health Score										
< mean	121	55.3	287	21.3	-10.03 (<0.0001)	359	56.8	630	28.1	-12.55 (<0.0001)
≥ mean	98	44.7	1,059	78.7		273	43.2	1,610	71.9	
Missing	4		38			11		67		
Mean (SE)	44.78 (0.55)		53.38 (0.22)		14.41 (<0.0001)	44.11 (0.38)		51.74 (0.20)		18.03 (<0.0001)

Table 2.32 (Continued): Characteristics of MDD cases and controls, by sex, AHP study, 1999.

	Males					Females				
	Cases		Controls		Chi-Square statistic (p-value)*	Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%		No.	%	No.	%	
Physical Health Score										
< mean	70	32.0	287	21.3	-3.35 (0.0008)	221	35.0	558	24.9	-4.46 (<0.0001)
≥ mean	149	68.0	1,059	78.7		411	65.0	1,682	75.1	
Missing	4		38			11		67		
Mean (SE)	50.42 (0.58)		51.89 (0.25)		2.37 (0.0189)	49.66 (0.38)		51.32 (0.21)		3.89 (0.0001)

Table 2.33: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for major depressive disorder, AHP study, 1999 (sensitivity analysis).

	Cases (866)		Controls (3,692)		OR	95% CI
	No.	%	No.	%		
Age at interview (years)						
< 36 years old	223	25.8	898	24.3	1.22	0.97, 1.53
36-43 years old	242	28.0	959	26.0	1.20	0.95, 1.50
44-51 years old	231	26.7	977	26.5	1.16	0.92, 1.46
> 51 years old	169	19.5	857	23.2	1.0	
Sex						
Male	223	25.8	2,307	62.5	1.0	
Female	643	74.2	1,384	37.5	1.72	1.45, 2.05
IBS						
Yes	69	8.0	143	3.9	1.95	1.44, 2.64
No	792	92.0	3,524	96.1	1.0	
CWP						
Yes	123	14.2	240	6.5	2.30	1.81, 2.92
No	742	85.8	3,445	93.5	1.0	
CFS-like illness						
Yes	94	10.9	68	1.8	5.76	4.17, 7.97
No	772	89.1	3,624	98.2	1.0	
BMI						
≤24.9 kg/m^2	367	42.4	1,619	43.9	1.0	
25-29.9 kg/m^2	268	30.9	1,241	33.6	0.96	0.80, 1.15
≥30 kg/m^2	231	26.7	832	22.5	1.26	1.04, 1.54

Table 2.33 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for major depressive disorder, AHP study, 1999 (sensitivity analysis).

	Cases (866)		Controls (3,692)		OR	95% CI
	No.	%	No.	%		
Mental Health Score						
< mean	480	56.4	917	25.6	3.69	3.14, 4.33
≥ mean	371	43.6	2,670	74.4	1.0	
Physical Health Score						
< mean	291	34.2	846	23.6	1.66	1.41, 1.96
≥ mean	560	65.8	2,741	76.4	1.0	
Continuous Covariates						
Age					0.99	0.99, 1.00
Mental Health Score					0.93	0.92, 0.94
Physical Health Score					0.98	0.97, 0.99

Table 2.34: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for major depressive disorder, by sex, AHP study, 1999.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age at interview (years)												
< 36 years old	38	17.0	280	20.2	0.89	0.56, 1.43	185	28.8	618	26.8	1.20	0.91, 1.57
36-43 years old	62	27.8	328	23.7	1.23	0.81, 1.85	180	28.0	631	27.4	1.10	0.83, 1.44
44-51 years old	68	30.5	390	28.2	1.18	0.80, 1.74	163	25.4	587	25.4	1.11	0.84, 1.47
> 51 years old	55	24.7	386	27.9	1.0		114	17.8	471	20.4	1.0	
IBS												
Yes	9	4.1	37	2.7	1.61	0.79, 3.28	60	9.4	106	4.6	1.89	1.35, 2.65
No	213	95.9	1,343	97.3	1.0		579	90.6	2,180	95.4	1.0	
CWP												
Yes	25	11.3	78	5.7	1.96	1.20, 3.20	98	15.2	162	7.0	2.32	1.76, 3.06
No	197	88.7	1,302	94.3	1.0		545	84.8	2,142	93.0	1.0	
CFS-like illness												
Yes	16	7.2	12	0.9	8.62	4.07, 18.22	78	12.1	56	2.4	4.89	3.41, 7.01
No	207	92.8	1,372	99.1	1.0		565	87.9	2,251	97.6	1.0	
BMI												
≤24.9 kg/m^2	69	30.9	463	33.4	1.0		298	46.3	1,156	50.1	1.0	
25-29.9 kg/m^2	104	46.6	600	43.4	1.17	0.83, 1.65	164	25.5	640	27.7	1.00	0.80, 1.25
≥30 kg/m^2	50	22.4	321	23.2	1.10	0.74, 1.65	181	28.1	511	22.1	1.40	1.12, 1.76

Table 2.34 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for major depressive disorder, by sex, AHP study, 1999.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Mental Health Score												
< mean	121	55.3	287	21.3	4.67	3.46, 6.31	359	56.8	630	28.1	3.23	2.68, 3.90
≥ mean	98	44.7	1,059	78.7	1.0		273	43.2	1,610	71.9	1.0	
Physical Health Score												
< mean	70	32.0	287	21.3	1.74	1.26, 2.39	221	35.0	558	24.9	1.58	1.30, 1.92
≥ mean	149	68.0	1,059	78.7	1.0		411	65.0	1,682	75.1	1.0	
Continuous Covariates												
Age					1.00	0.98, 1.01					1.00	0.99, 1.01
Mental Health Score					0.92	0.90, 0.93					0.93	0.93, 0.94
Physical Health Score					0.98	0.96, 1.00					0.98	0.97, 0.99

Table 2.35: Assessing effect measure modification for IBS-MDD model, AHP study, 1999 (Sensitivity Analysis).

	OR, (95% CI) for IBS	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age (years)		2.02 (1.49, 2.74)	4.3499 (0.2261)	Maybe
< 36 years old	2.43 (1.33, 4.43)			
36-43 years old	2.92 (1.68, 5.07)			
44-51 years old	1.34 (0.70, 2.54)			
> 51 years old	1.50 (0.72, 3.11)			
Sex		1.89 (1.39, 2.58)	0.2267 (0.6340)	Maybe
Male	1.61 (0.76, 3.39)			
Female	1.96 (1.39, 2.75)			
CWP		1.75 (1.28, 2.38)	2.6681 (0.1024)	Yes
Yes	1.12 (0.59, 2.12)			
No	2.05 (1.44, 2.91)			
CFS-like illness		1.57 (1.15, 2.15)	15.0354 (0.0001)	Yes
Yes	0.39 (0.17, 0.89)			
No	2.11 (1.51, 2.95)			
BMI		2.00 (1.47, 2.72)	0.0617 (0.9696)	No
≤24.9 kg/m^2	2.01 (1.21, 3.34)			
25-29.9 kg/m^2	1.90 (1.11, 3.25)			
≥30 kg/m^2	2.10 (1.20, 3.66)			
Mental Health Score		1.66 (1.21, 2.28)	0.2849 (0.5935)	Maybe
< mean	1.55 (1.02, 2.35)			
≥ mean	1.85 (1.13, 3.02)			
Physical Health Score		1.77 (1.30, 2.41)	2.2758 (0.1314)	Yes
< mean	1.42 (0.91, 2.19)			
≥ mean	2.27 (1.47, 3.51)			

Table 2.36: Evaluation of confounding for the association between lifetime IBS and risk of lifetime MDD, AHP study, 1999.

	Lifetime IBS and covariate relationship	Relationship between covariate and lifetime MDD	Adjusted OR	Ln[CoRR]
OR (95% CI) for lifetime IBS 1.95 (1.44, 2.64)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	Ln(OR_{crude} /OR_{adj})
Total=4,307	3,481 (controls)	4,104 (no lifetime IBS)		
Age (years)				
Continuous	1.00 (0.98, 1.01)	1.00 (0.99, 1.00)	1.94 (1.44, 2.63)	0.005
< 36 years old	1.04 (0.68, 1.60)	1.18 (0.93, 1.49)		
36-43 years old	1.13 (0.75, 1.71)	1.13 (0.90, 1.44)		
44-51 years old	1.06 (0.70, 1.61)	1.17 (0.93, 1.48)		
> 51 years old	1.0		1.94 (1.44, 2.63)	0.005
Sex				
Male	1.0			
Female	2.03 (1.44, 2.85)	1.68 (1.40, 2.01)	1.84 (1.36, 2.49)	0.058
CWP				
Yes	3.89 (2.74, 5.53)	2.35 (1.82, 3.04)	1.72 (1.25, 2.35)	0.126
No	1.0			
CFS-like illness				
Yes	5.13 (3.30, 7.97)	7.23 (5.01, 10.41)	1.61 (1.15, 2.26)	0.192
No	1.0			
BMI	Categorical			
≤24.9 kg/m^2	1.0		1.93 (1.42, 2.61)	0.010
25-29.9 kg/m^2	1.17 (0.83, 1.64)	0.96 (0.79, 1.15)		
≥30 kg/m^2	1.40 (0.98, 2.00)	1.25 (1.02, 1.52)		
Mental Health Score				
Continuous	0.97 (0.95, 0.98)	0.93 (0.92, 0.94)	1.50 (1.08, 2.09)	0.262
< mean	2.09 (1.56, 2.78)	3.68 (3.12, 4.35)		
≥ mean	1.0		1.62 (1.18, 2.22)	0.185
Physical Health Score				
Continuous	0.95 (0.94, 0.97)	0.98 (0.97, 0.99)	1.76 (1.29, 2.39)	0.103
< mean	3.20 (2.41, 4.26)	1.66 (1.40, 1.97)		
≥ mean	1.0		1.73 (1.27, 2.36)	0.120

Table 2.37: Assessing confounding and effect measure modification using backward elimination for the model of lifetime IBS, AHP study, 1999.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**
N (cases/controls)	4,307 (826/3,481)	4,307 (826/3,481)	4,307 (826/3,481)
Main Exposure:			
Lifetime IBS	0.6666 (0.1548)	2.0263 (0.9940)	0.5921 (0.1548)
|ln(crude/adj)| of beta		1.11	1.23
Compare to:		Min Model	Full
-2log L	4191.9522	4141.2142	4144.5022
df	1	7	4
Chi-square p-value			3.288 (p>0.2)
OR (95% CI) for main effect:			
lifetime IBS	1.95 (1.44, 2.64)	7.59 (1.08, 53.23)	1.81 (1.33, 2.45)
Crude vs Adjusted |ln(crude/adj)|		1.36	1.43
Notes/Conclusions about current model:	IBS, adjusted for twin relatedness.	First-order interactions with gender, BMI, and age, and linear terms.	All interaction terms excluded from model to assess effect modification. No effect modification is present based on likelihood ratio test.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	Age (0.2886)

Table 2.37 (Continued): Assessing confounding and effect measure modification using backward elimination for the model of lifetime IBS, AHP study, 1999.

	Model 3 **β (SE)**	**Model 4** **β (SE)**	**MODEL 5/FINAL MODEL** **β (SE)**
N (cases/controls)	4,307 (826/3,481)	4,307 (826/3,481)	4,307 (826/3,481)
Main Exposure:			
Lifetime IBS	0.5932 (0.1552)	0.6075 (0.1549)	0.6666 (0.1548)
|ln(crude/adj)| of beta	0.002	0.02	0.09
Compare to:	Model 2	Model 3	Model 4
-2log L	4145.6714	4152.8428	4191.9522
df	3	2	1
Chi-square p-value	N/A	N/A	N/A
OR (95% CI) for main effect:			
lifetime IBS	1.81 (1.34, 2.45)	1.84 (1.36, 2.49)	1.95 (1.44, 2.64)
Crude vs Adjusted			
|ln(crude/adj)|	0	0.02	0.06
Notes/Conclusions about current model:	OR for main effect did not change by >10%. Age is not confounder.	OR for main effect did not change by >10%. BMI is not confounder.	OR for main effect is not confounded by any potential covariates, but is adjusted for twin relatedness.
Variable to be dropped next (Wald p-value)	BMI (0.0288)	Gender (<0.0001)	

Table 2.38: Characteristics of IBS cases and controls, SALT study, 2002.

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
Total	29,616		611	2.06	29,005	97.94	
Age at interview (years)							25.9913 (0.0005)
42-44	2,494	8.42	68	11.13	2,426	8.36	
45-47	3,796	12.82	91	14.89	3,705	12.77	
48-50	3,727	12.58	97	15.88	3,630	12.52	
51-53	4,063	13.72	93	15.22	3,970	13.69	
54-56	7,221	24.38	131	21.44	7,090	24.44	
57-59	3,743	12.64	60	9.82	3,683	12.70	
60-62	3,033	10.24	48	7.86	2,985	10.29	
63+	1,539	5.20	23	3.76	1,516	5.23	
Sex							
Male	14,187	47.9	221	36.17	13,966	48.15	-5.56 (<0.0001)
Female	15,429	52.1	390	63.83	15,039	51.85	
MDD							
Yes	6,502	21.95	275	45.01	6,227	21.47	-12.84 (<0.0001)
No	23,114	78.05	336	54.99	22,778	78.53	
CWP							
Yes	760	2.57	69	11.29	691	2.38	-12.01 (<0.0001)
No	28,856	97.43	542	88.71	28,314	97.62	
CF-like illness							
Yes	669	2.26	72	11.78	597	2.06	-13.75 (<0.0001)
No	28,947	97.74	539	88.22	28,408	97.94	

Table 2.38 (Continued): Characteristics of IBS cases and controls, SALT study, 2002.

	Total		Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%	No.	%	
BMI							
<18.5	349	1.18	12	1.96	337	1.16	13.3592 (0.0039)
18.5-24.9 kg/m^2	15,561	52.54	316	51.72	15,245	52.56	
25-29.9 kg/m^2	11,246	37.97	212	34.70	11,034	38.04	
≥30 kg/m^2	2,460	8.31	71	11.62	2,389	8.24	
Health compared to 5 years ago							
Better	22,830	77.09	361	59.08	22,469	77.47	10.27 (<0.0001)
Worse	6,786	22.91	250	40.92	6,536	22.53	
Does health limit your activities?							
Not limited	21,973	74.19	306	50.08	21,667	74.70	12.96 (<0.0001)
Limited	7,643	25.81	305	49.92	7,338	25.30	
Number of days health limited activities							
> a week	3,549	11.98	144	23.57	3,405	11.74	8.54 (<0.0001)
< a week	26,067	88.02	467	76.43	25,600	88.26	

Table 2.39: Characteristics of IBS cases and controls, by sex, SALT study, 2002.

	Males					Females				
	Cases		Controls		Chi-Square statistic (p-value)*	Cases		Controls		Chi-Square statistic (p-value)*
	No.	%	No.	%		No.	%	No.	%	
Total	221	1.6	13,966	98.4		390	2.5	15,039	97.5	
Age at interview (years)					17.8124 (0.0128)					13.3388 (0.0643)
42-44	26	11.8	1,166	8.3		42	10.8	1,260	8.4	
45-47	40	18.1	1,814	13.0		51	13.1	1,891	12.6	
48-50	34	15.4	1,778	12.7		63	16.2	1,852	12.3	
51-53	35	15.8	1,921	13.8		58	14.9	2,049	13.6	
54-56	43	19.5	3,367	24.1		88	22.6	3,723	24.8	
57-59	22	9.9	1,762	12.6		38	9.7	1,921	12.8	
60-62	12	5.4	1,405	10.1		36	9.2	1,580	10.5	
63+	9	4.1	753	5.4		14	3.6	763	5.1	
MDD					-6.43 (<0.0001)					-10.08 (<0.0001)
Yes	69	31.2	2,037	14.6		206	52.8	4,190	27.9	
No	152	68.8	11,929	85.4		184	47.2	10,849	72.1	
CWP					-3.97 (<0.0001)					-10.24 (<0.0001)
Yes	8	3.6	113	0.8		61	15.6	578	3.8	
No	213	96.4	13,853	99.2		329	84.4	14,461	96.2	
CF-like illness					-5.74 (<0.0001)					-11.51 (<0.0001)
Yes	11	5.0	120	0.9		61	15.6	477	3.2	
No	210	95.0	13,846	99.1		329	84.4	14,562	96.8	

Table 2.39 (Continued): Characteristics of IBS cases and controls, by sex, SALT study, 2002.

	Males					Females				
	Cases		Controls		Chi-Square	Cases		Controls		Chi-Square
	No.	%	No.	%	statistic (p-value)*	No.	%	No.	%	statistic (p-value)*
BMI					10.8808 (0.0124)					7.2821 (0.0634)
<18.5	0	0	47	0.3		12	3.1	290	1.9	
18.5-24.9 kg/m^2	100	45.2	6,123	43.8		216	55.4	9,122	60.7	
25-29.9 kg/m^2	89	40.3	6,568	47.0		123	31.5	4,466	29.7	
≥30 kg/m^2	32	14.5	1,228	8.8		39	10.0	1,161	7.7	
Health compared to 5 years ago					6.17 (<0.0001)					7.94 (<0.0001)
Better	135	61.1	11,037	79.0		226	57.9	11,432	76.0	
Worse	86	38.9	2,929	21.0		164	42.1	3,607	24.0	
Does health limit your activities?					6.93 (<0.0001)					10.52 (<0.0001)
Not limited	125	56.6	10,808	77.4		181	46.4	10,859	72.2	
Limited	96	43.4	3,158	22.6		209	53.6	4,180	27.8	
Number of days health limited activities					3.28 (0.0010)					7.48 (<0.0001)
> a week	36	16.3	1,336	9.6		108	27.7	2,069	13.8	
< a week	185	83.7	12,630	90.4		282	72.3	12,970	86.2	

Table 2.40: Association between IBS status and missing covariate information, SALT study, 2002.

	Case (647)		Control (30,524)		χ^2	p-value
	No.	%	No.	%		
Major Depressive Disorder						
Missing	20	3.09	622	2.04	3.4856	0.0619
Not missing	627	96.91	29,902	97.96		
Chronic Widespread Pain						
Missing	5	0.77	59	0.19	10.38	0.0013
Not missing	642	99.23	30, 465	99.81		
Gender						
Missing	0	0	0	0	0	1.0
Not missing	647	100	30,524	100		
Health compared to 5 years ago						
Missing	1	0.15	63	0.21	0.0831	0.7732
Not missing	646	99.85	30,461	99.79		
Does health limit your activities?						
Missing	1	0.15	57	0.19	0.0353	0.8509
Not missing	646	99.85	30,467	99.81		
Number of days health limited activities						
Missing	0	0	0	0	0	1.0
Not missing	647	100	30,524	100		
Chronic Fatigue-like illness						
Missing	1	0.15	180	0.59	2.08	0.1494
Not missing	646	99.85	30,344	99.41		
Body Mass Index						
Missing	0	0	0	0	0	1.0
Not missing	647	100	30,524	100		

Table 2.41: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, SALT study, 2002.

	Cases (611)		Controls (29,005)		OR	95% CI
	No.	%	No.	%		
Age at interview (years)						
42-44	68	11.13	2,426	8.36	1.85	1.15, 2.97
45-47	91	14.89	3,705	12.77	1.62	1.02, 2.57
48-50	97	15.88	3,630	12.52	1.77	1.12, 2.79
51-53	93	15.22	3,970	13.69	1.54	0.98, 2.44
54-56	131	21.44	7,090	24.44	1.22	0.78, 1.90
57-59	60	9.82	3,683	12.70	1.08	0.67, 1.75
60-62	48	7.86	2,985	10.29	1.06	0.64, 1.75
63+	23	3.76	1,516	5.23	1.0	Referent
Sex						
Female	390	63.83	15,039	51.85	1.64	1.39, 1.93
Male	221	36.17	13,966	48.15		
MDD						
Yes	275	45.01	6,227	21.47	2.98	2.53, 3.51
No	336	54.99	22,778	78.53		
CWP						
Yes	69	11.29	691	2.38	5.17	3.97, 6.73
No	542	88.71	28,314	97.62		
CF-like illness						
Yes	72	11.8	597	2.1	6.31	4.87, 8.18
No	539	88.2	28,408	97.9		
BMI						
<18.5	12	1.96	337	1.16	1.74	0.97, 3.12
18.5-24.9 kg/m^2	316	51.72	15,245	52.56	1.0	Referent
25-29.9 kg/m^2	212	34.70	11,034	38.04	0.93	0.78, 1.11
≥30 kg/m^2	71	11.62	2,389	8.24	1.43	1.10, 1.87

Table 2.41 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, SALT study, 2002.

	Cases (611)		Controls (29,005)		OR	95% CI
	No.	%	No.	%		
Health compared to 5 years ago						
Better	361	59.08	22,469	77.47	0.42	0.36, 0.50
Worse	250	40.92	6,536	22.53		
Does health limit your activities?						
Not limited	306	50.08	21,667	74.70	0.34	0.29, 0.40
Limited	305	49.92	7,338	25.30		
Number of days health limited activities						
> a week	144	23.57	3,405	11.74	0.43	0.36, 0.52
< a week	467	76.43	25,600	88.26		

Table 2.42: Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, by sex, SALT study, 2002.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age at interview (years)												
42-44	26	11.8	1,166	8.3	1.86	0.87, 3.98	42	10.8	1,260	8.4	1.83	0.99, 3.36
45-47	40	18.1	1,814	13.0	1.84	0.89, 3.82	51	13.1	1,891	12.6	1.39	0.82, 2.69
48-50	34	15.4	1,778	12.7	1.60	0.76, 3.36	63	16.2	1,852	12.3	1.87	1.05, 3.35
51-53	35	15.8	1,921	13.8	1.52	0.73, 3.17	58	14.9	2,049	13.6	1.56	0.87, 2.79
54-56	43	19.5	3,367	24.1	1.07	0.52, 2.20	88	22.6	3,723	24.8	1.30	0.74, 2.29
57-59	22	9.9	1,762	12.6	1.04	0.48, 2.28	38	9.7	1,921	12.8	1.09	0.59, 2.01
60-62	12	5.4	1,405	10.1	0.71	0.30, 1.69	36	9.2	1,580	10.5	1.25	0.67, 2.33
63+	9	4.1	753	5.4	1.0	Referent	14	3.6	763	5.1	1.0	Referent
MDD												
Yes	69	31.2	2,037	14.6	2.65	1.99, 3.55	206	52.8	4,190	27.9	2.89	2.36, 3.55
No	152	68.8	11,929	85.4			184	47.2	10,849	72.1		
CWP												
Yes	8	3.6	113	0.8	4.54	2.16, 9.53	61	15.6	578	3.8	4.62	3.46, 6.15
No	213	96.4	13,853	99.2			329	84.4	14,461	96.2		
CF-like illness												
Yes	11	5.0	120	0.9	6.08	3.24, 11.43	61	15.6	477	3.2	5.62	4.21, 7.51
No	210	95.0	13,846	99.1			329	84.4	14,562	96.8		
BMI												
<18.5	0	0	47	0.3			12	3.1	290	1.9	1.77	0.98, 3.19
18.5-24.9 kg/m^2	100	45.2	6,123	43.8	1.0	Referent	216	55.4	9,122	60.7	1.0	Referent
25-29.9 kg/m^2	89	40.3	6,568	47.0	0.83	0.62, 1.11	123	31.5	4,466	29.7	1.16	0.93, 1.46
≥30 kg/m^2	32	14.5	1,228	8.8	1.60	1.07, 2.39	39	10.0	1,161	7.7	1.42	0.99, 2.03

Table 2.42 (Continued): Odds ratios (OR) and 95% confidence intervals (CI) for the association between selected risk factors and risk for irritable bowel syndrome, by sex, SALT study, 2002.

	Males						Females					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	No.	%	No.	%			No.	%	No.	%		
Health compared to 5 years ago												
Better	135	61.1	11,037	79.0	0.42	0.32, 0.55	226	57.9	11,432	76.0	0.44	0.35, 0.53
Worse	86	38.9	2,929	21.0			164	42.1	3,607	24.0		
Does health limit your activities?												
Not limited	125	56.6	10,808	77.4	0.38	0.29, 0.50	181	46.4	10,859	72.2	0.33	0.27, 0.41
Limited	96	43.4	3,158	22.6			209	53.6	4,180	27.8		
Number of days health limited activities												
> a week	36	16.3	1,336	9.6	0.55	0.38, 0.78	108	27.7	2,069	13.8	0.42	0.33, 0.52
< a week	185	83.7	12,630	90.4			282	72.3	12,970	86.2		

Table 2.43: Assessing effect measure modification for MDD-IBS model, SALT study, 2002.

	OR, (95% CI) for MDD	OR_{M-H} (95% CI)	Breslow-Day test statistic (p-value)	Effect Modifier (Yes/No/ Maybe)
Age at interview (years)		2.92 (2.49, 3.44)	4.2838 (0.7466)	No
42-44	3.05 (1.88, 4.95)			
45-47	3.53 (2.32, 5.36)			
48-50	2.50 (1.67, 3.76)			
51-53	3.09 (2.04, 4.67)			
54-56	2.78 (1.96, 3.95)			
57-59	3.75 (2.24, 6.28)			
60-62	1.89 (1.01, 3.55)			
63+	2.97 (1.24, 7.08)			
Sex		2.83 (2.39, 3.33)	0.2337 (0.6288)	No
Female	2.90 (2.37, 3.55)			
Male	2.66 (1.99, 3.55)			
CWP		2.71 (2.30, 3.19)	8.1131 (0.0044)	Yes
Yes	1.41 (0.86, 2.31)			
No	2.98 (2.51, 3.54)			
CF-like illness		2.63 (2.23, 3.11)	3.2720 (0.0705)	Yes
Yes	1.72 (1.03, 2.85)			
No	2.80 (2.36, 3.33)			
BMI		2.97 (2.53, 3.49)	2.4854 (0.4779)	Maybe
<18.5	1.99 (0.62, 6.43)			
18.5-24.9 kg/m^2	3.03 (2.42, 3.79)			
25-29.9 kg/m^2	3.28 (2.49, 4.31)			
≥30 kg/m^2	2.19 (1.35, 3.56)			
Health compared to 5 years ago		2.70 (2.30, 3.18)	10.4088 (0.0013)	Yes
Better	3.42 (2.78, 4.23)			
Worse	1.99 (1.55, 2.57)			
Does health limit your activities?		2.41 (2.12, 2.94)	8.8540 (0.0029)	Yes
Not limited	3.26 (2.59, 4.10)			
Limited	2.00 (1.59, 2.51)			
Number of days health limited activities		2.78 (2.36, 3.28)	0.1731 (0.6774)	No
> a week	2.96 (2.10, 4.16)			
< a week	2.72 (2.26, 3.28)			

Table 2.44: Evaluation of confounding for the association between lifetime MDD and risk of lifetime IBS, SALT study, 2002.

	Lifetime MDD and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for lifetime MDD 2.98 (2.53, 3.51)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=29,616	29,005 (controls)	23,114 (no lifetime MDD)		
Age at interview (years)			2.91 (2.47, 3.43)	0.0238
42-44	1.74 (1.46, 2.06)	1.61 (0.88, 2.96)		
45-47	1.78 (1.51, 2.09)	1.30 (0.72, 2.37)		
48-50	1.77 (1.51, 2.08)	1.68 (0.95, 2.99)		
51-53	1.70 (1.45, 1.99)	1.35 (0.75, 2.41)		
54-56	1.53 (1.32, 1.79)	1.14 (0.65, 2.00)		
57-59	1.22 (1.03, 1.44)	0.94 (0.51, 1.73)		
60-62	1.19 (1.00, 1.41)	1.19 (0.64, 2.19)		
63+	1.0	1.0		
Sex			2.80 (2.37, 3.31)	0.0623
Female	2.29 (2.16, 2.43)	1.33 (1.07, 1.65)		
Male	1.0	1.0		
CWP			2.74 (2.32, 3.24)	0.0840
Yes	2.88 (2.49, 3.34)	6.25 (4.30, 9.08)		
No	1.0	1.0		
CF-like illness			2.66 (2.25, 3.15)	0.1136
Yes	3.86 (3.30, 4.51)	6.38 (4.23, 9.64)		
No	1.0	1.0		
BMI			2.99 (2.54, 3.52)	0.0034
<18.5	1.24 (0.97, 1.57)	2.00 (0.93, 4.30)		
18.5-24.9 kg/m^2	1.0	1.0		
25-29.9 kg/m^2	0.84 (0.79, 0.89)	0.94 (0.74, 1.19)		
≥30 kg/m^2	1.03 (0.93, 1.14)	1.64 (1.16, 2.32)		

Table 2.44 (Continued): Evaluation of confounding for the association between lifetime MDD and risk of lifetime IBS, SALT study, 2002.

	Lifetime MDD and covariate relationship	Relationship between covariate and lifetime IBS	Adjusted OR	Ln[CoRR]
OR (95% CI) for lifetime MDD 2.98 (2.53, 3.51)	OR, (95% CI)	OR, (95% CI)	OR, (95% CI)	$Ln(OR_{crude}/OR_{adj})$
Total=29,616	29,005 (controls)	23,114 (no lifetime MDD)		
Health compared to 5 years ago			2.73 (2.31, 3.23)	0.0876
Better	0.58 (0.55, 0.62)	0.37 (0.30, 0.46)		
Worse	1.0	1.0		
Does health limit your activities?			2.53 (2.14, 3.00)	0.1637
Not limited	0.46 (0.44, 0.49)	0.32 (0.26, 0.40)		
Limited	1.0	1.0		
Number of days health limited activities			2.76 (2.34, 3.26)	0.0767
> a week	1.0	1.0		
< a week	0.46 (0.43, 0.50)	0.54 (0.40, 0.72)		

Table 2.45: Assessing confounding and effect measure modification in the case-control study, using backward elimination for the model of lifetime MDD, SALT study, 2002.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**	**Model 3/FINAL MODEL β (SE)**
N (cases/controls)	29,616 (611/29,605)	29,616 (611/29,605)	29,616 (611/29,605)	29,616 (611/29,605)
Main Exposure: Lifetime MDD	1.0052 (0.0847)	0.7083 (0.6767)	1.0007 (0.0848)	1.0052 (0.0847)
|ln(crude/adj)| of beta		0.35	0.35	0.004
Compare to:		Min Model	Full	Full
-2log L	5755.3826	5748.2854	5748.6312	5755.3826
df	3	6	4	3
Chi-square p-value			0.3458 (p>0.2)	N/A
OR (95% CI) for main effect: lifetime MDD	2.73 (2.31, 3.23)	2.03 (0.54, 7.65)	2.72 (2.30, 3.21)	2.73 (2.31, 3.23)
Crude vs Adjusted |ln(crude/adj)|		0.30	0.29	0.004
Notes/Conclusions about current model:	MDL, adjusted for twin relatedness, sex, and 3-year age group.	First-order interactions with gender and BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. Effect modification is present based on likelihood ratio test.	OR for main effect did not change by >10%. BMI is not a confounder. OR for main effect is not confounded by any covariates, but it is adjusted for twin relatedness, 3-year age group, and sex.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.0126)	None

Table 2.46: Assessing confounding and effect measure modification in the co-twin control study (MZ and DZ IBS discordant twin pairs), using backward elimination for the model of lifetime MDD, SALT study, 2002.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**	**Model 3/FINAL MODEL β (SE)**
# IBS discordant pairs	288	288	288	288
Main Exposure:				
Lifetime MDD	0.7985 (0.2007)	0.9679 (0.2491)	0.7975 (0.2010)	0.7985 (0.2007)
|ln(crude/adj)| of beta		0.19	0.19	0.001
Compare to:		Min Model	Full	Full
-2log L	382.138	379.776	381.220	382.138
df	1	3	2	1
Chi-square p-value			1.444 (p>0.2)	N/A
OR (95% CI) for main effect:				
lifetime MDD	2.22 (1.50, 3.29)	2.63 (1.62, 4.29)	2.22 (1.50, 3.29)	2.22 (1.50, 3.29)
Crude vs Adjusted				
|ln(crude/adj)|		0.17	0.17	0
Notes/Conclusions about current model:	MDL only.	First-order interaction with BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. Effect modification is present based on likelihood ratio test.	OR for main effect did not change by >10%. BMI is not a confounder. OR for main effect is not confounded by any covariates.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.3404)	None

Table 2.47: Assessing confounding and effect measure modification in the co-twin control study (MZ IBS discordant twin pairs), using backward elimination for the model of lifetime MDD, SALT study, 2002.

	Min Model β (SE)	**Full Model β (SE)**	**Model 2 β (SE)**	**Model 3/FINAL MODEL β (SE)**
# IBS discordant pairs	119	119	119	119
Main Exposure: Lifetime MDD	1.1527 (0.3311)	1.1811 (0.4606)	1.1556 (0.3317)	1.1527 (0.3311)
|ln(crude/adj)| of beta		0.02	0.02	0.003
Compare to:		Min Model	Full	Full
-2log L	150.762	150.729	150.736	150.762
df	1	3	2	1
Chi-square p-value			0.007 (p>0.2)	N/A
OR (95% CI) for main effect: lifetime MDD	3.17 (1.66, 6.06)	3.26 (1.32, 8.04)	3.18 (1.66, 6.09)	3.17 (1.66, 6.06)
Crude vs Adjusted |ln(crude/adj)|		0.03	0.03	0.003
Notes/Conclusions about current model:	MDL only.	First-order interaction with BMI, and linear terms.	All interaction terms excluded from model to assess effect modification. Effect modification is present based on likelihood ratio test.	OR for main effect did not change by >10%. BMI is not a confounder. OR for main effect is not confounded by any covariates.
Variable to be dropped next (Wald p-value)		All interaction terms (chunk test)	BMI (0.8703)	None

Table 2.48: Co-twin control analysis explanation.

Step 1: 'Classic' case-control study—assesses association between exposure and outcome	**Step 2: Healthy co-twin used as control for diseased twin (MZ and DZ twin pairs)**—controls for early environmental confounding	**Step3: Only disease discordant MZ twins used**—controls for unmeasured genetic background	**Results Interpretation**
Association demonstrated	Association demonstrated	Association demonstrated	Exposure contributes to the causation of the disease (Causal model)
Association demonstrated	Association demonstrated	No association demonstrated	Genetic effects confounded the results (Non-causal genetic model)
Association demonstrated	No association demonstrated	Association demonstrated	Perversity
Association demonstrated	No association demonstrated	No association demonstrated	Non-causal Family Environment Model
No association demonstrated	Not applicable	Not applicable	No association

Table 2.49: Summary of Power Calculations using the AHP data.

		Prevalence Odds Ratio Detected Using 203 IBS cases				
Exposure (MDD) Prevalence	Outcome (IBS) Prevalence	1.2	1.3	1.5	1.6	1.7
20%	5%	29%	47%	78%	88%	94%
	10%	28%	45%	76%	86%	93%
15%	5%	26%	42%	72%	82%	89%
	10%	25%	40%	69%	80%	88%
10%	5%	22%	34%	61%	72%	81%
	10%	21%	33%	59%	70%	79%

2.5 FIGURES

Figure 2.1: Directed acyclic graph to assess associations between MDD and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is age, gender, and BMI. CFS-like illness is abbreviated as CFS in this graph.

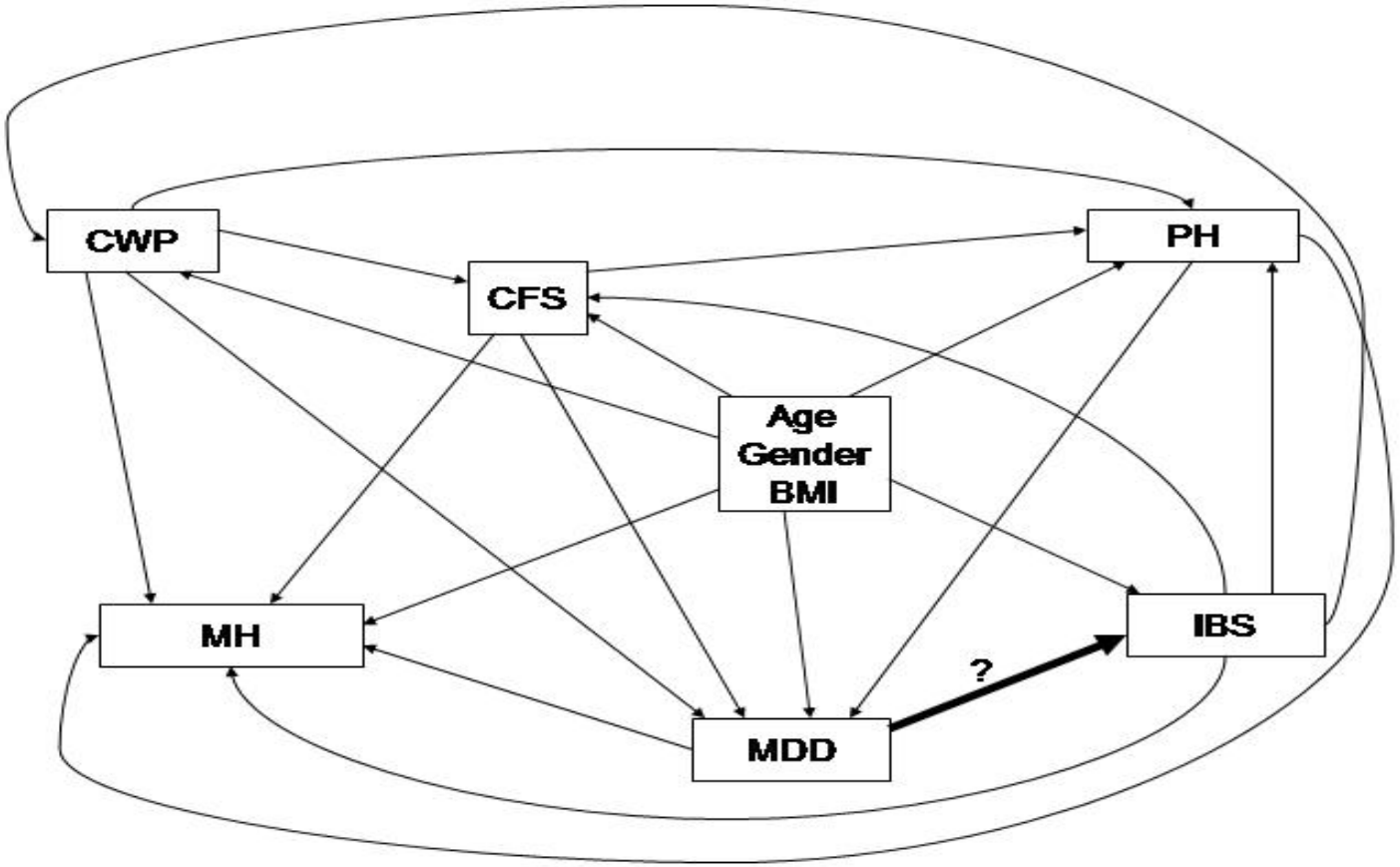


Figure 2.2: Directed acyclic graph to assess associations between gender and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is the null set. CFS-like illness is abbreviated as CFS in this graph.

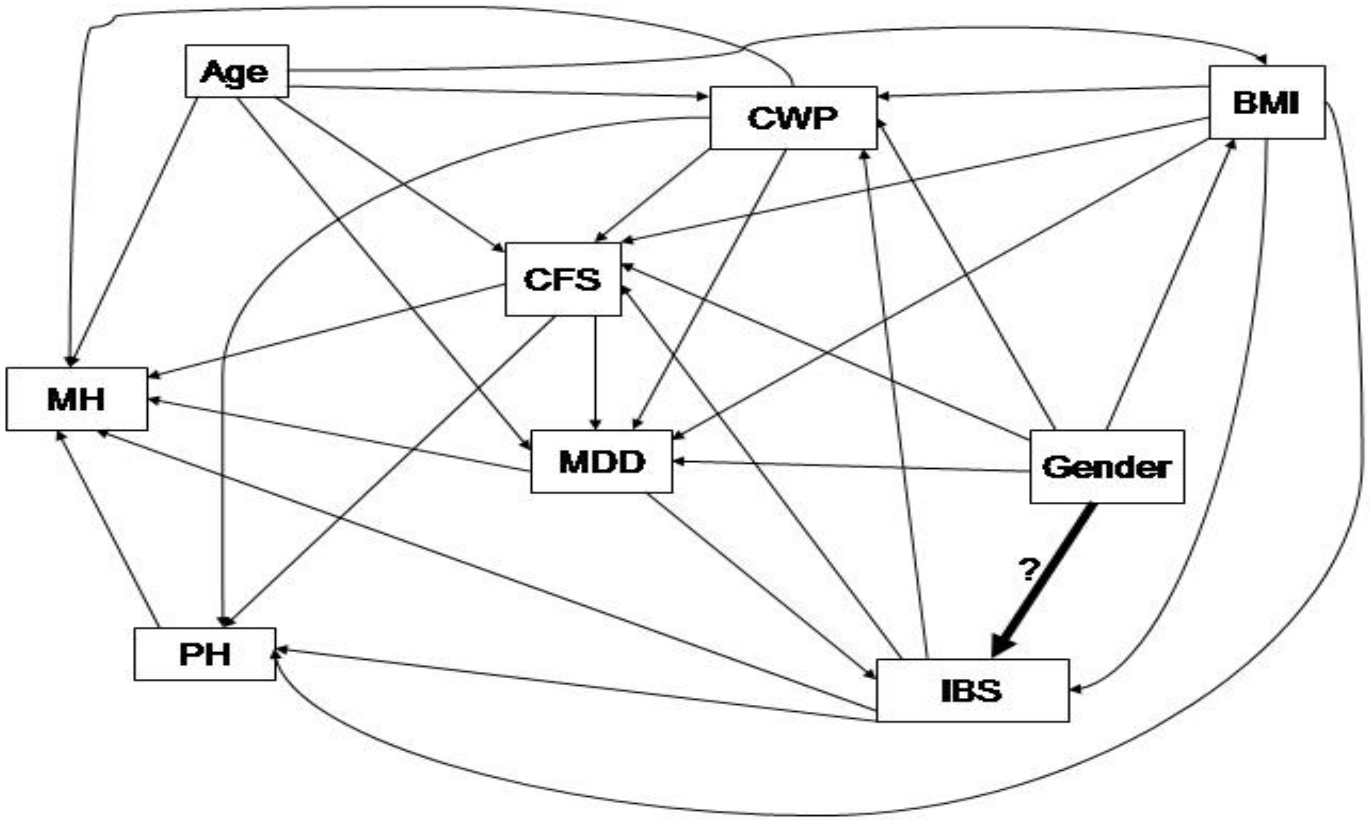


Figure 2.3: Directed acyclic graph to assess associations between CWP and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is age, gender, and BMI. CFS-like illness is abbreviated as CFS in this graph.

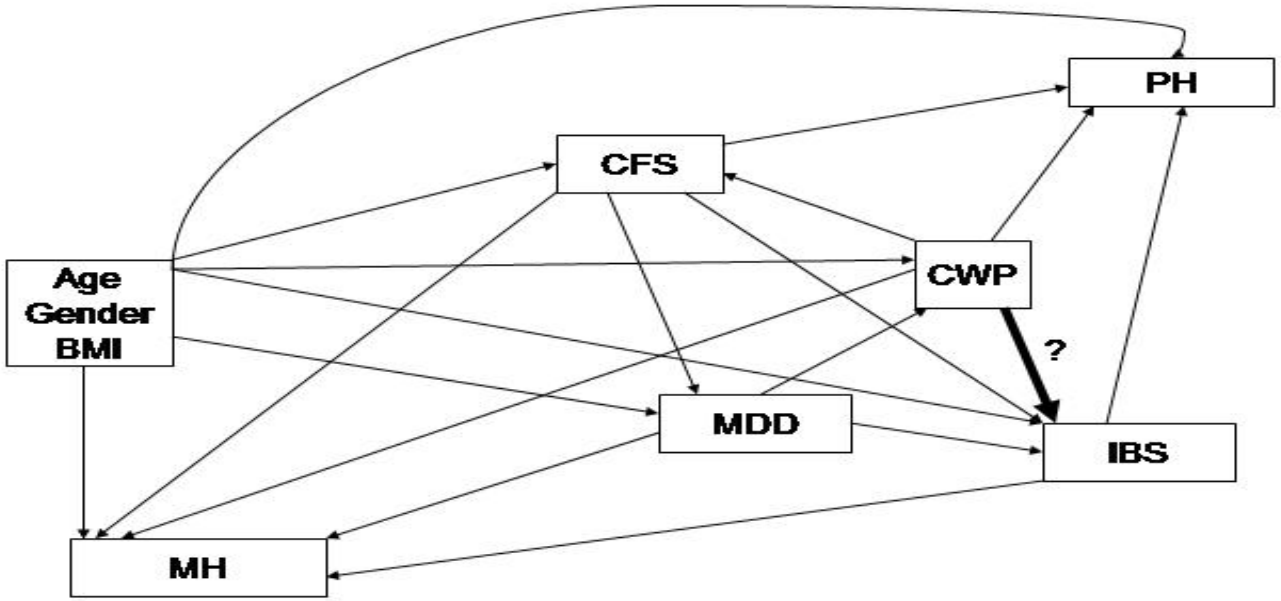


Figure 2.4: Directed acyclic graph to assess associations between CFS-like illness and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is age, gender, and BMI. CFS-like illness is abbreviated as CFS in this graph.

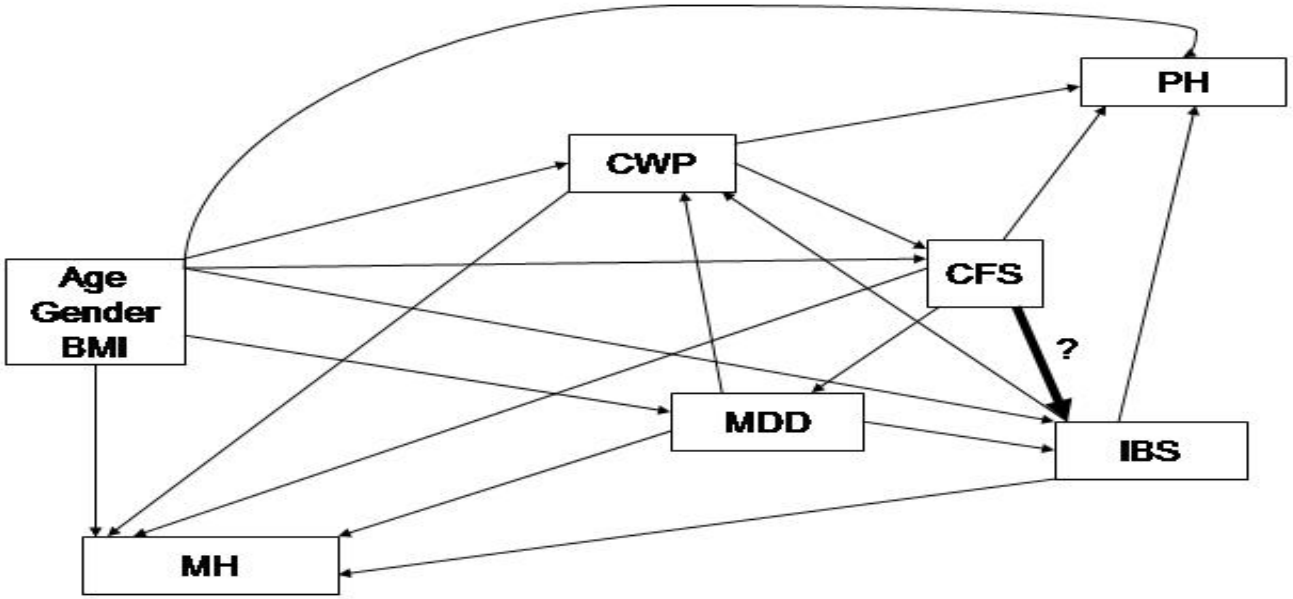


Figure 2.5: Directed acyclic graph to assess associations between age and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is the null set. CFS-like illness is abbreviated as CFS in this graph.

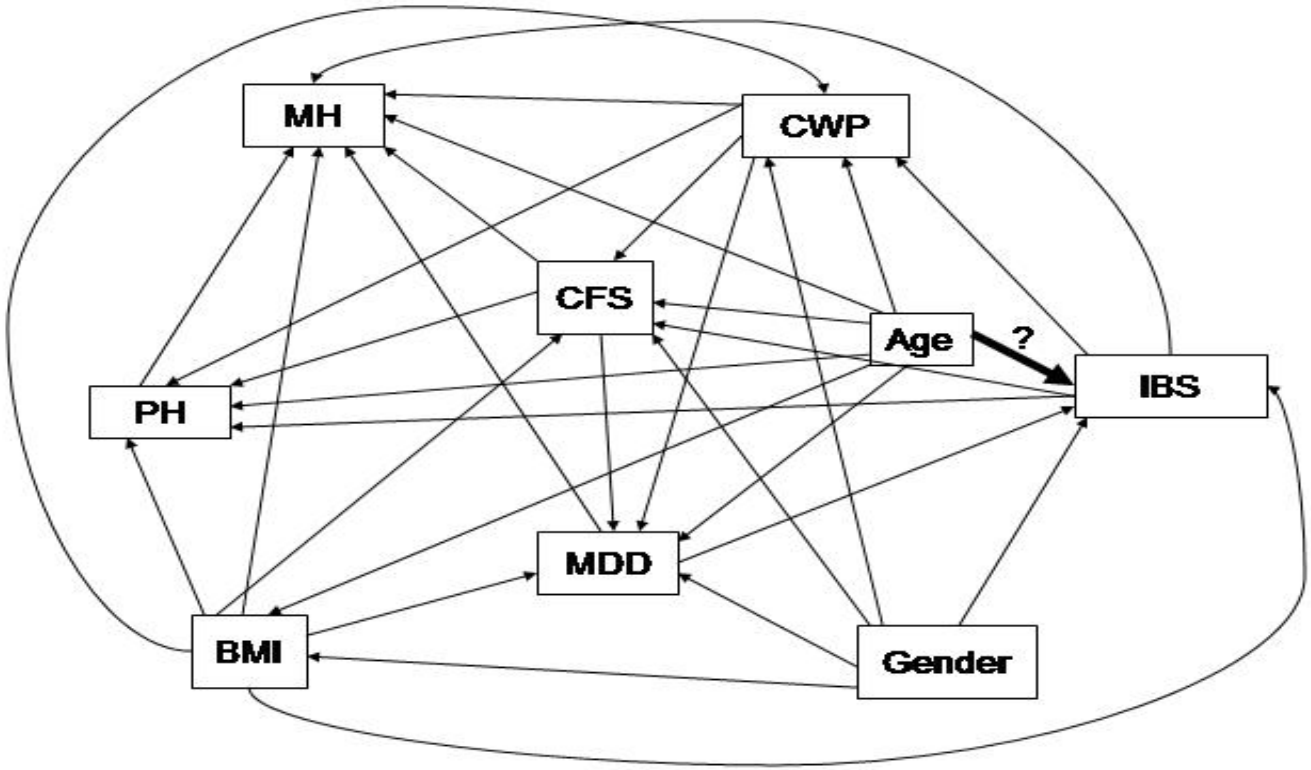


Figure 2.6: Directed acyclic graph to assess associations between BMI and IBS in the AHP study, 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is age and gender. CFS-like illness is abbreviated as CFS in this graph.

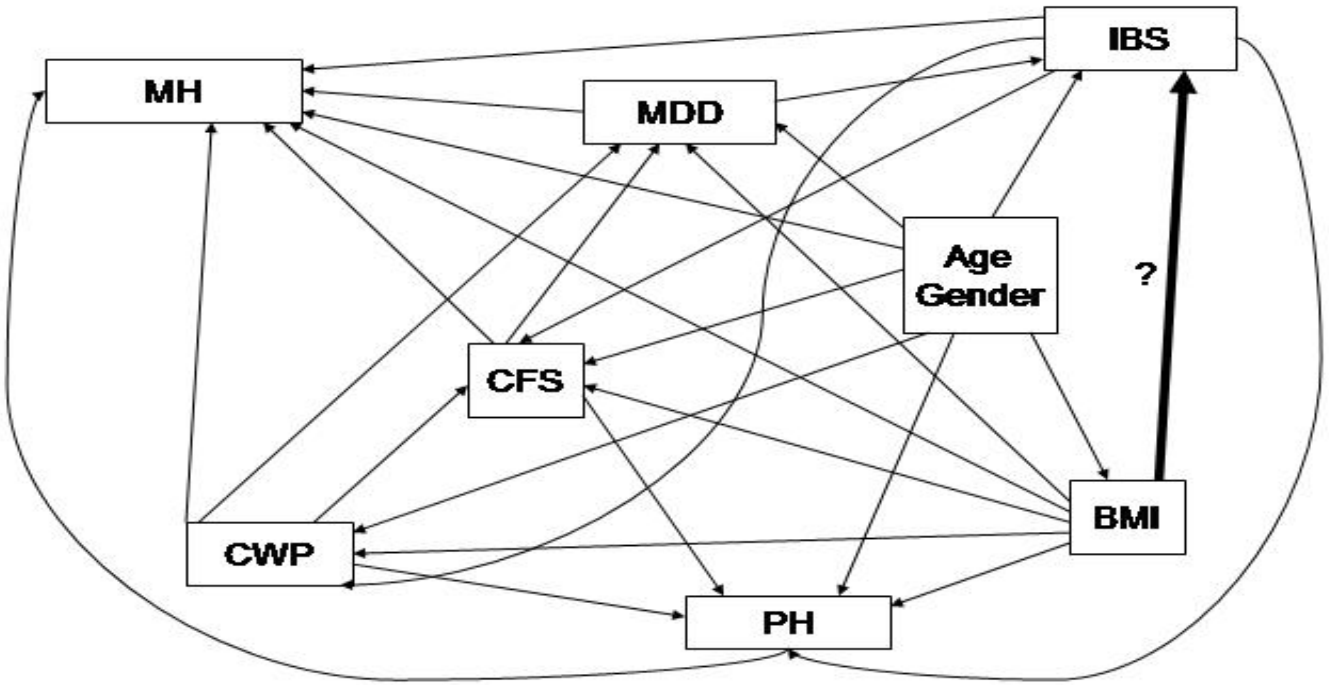


Figure 2.7: Directed acyclic graph to assess associations between IBS and MDD in the AHP study (sensitivity analysis), 1999. Analysis of this graph implies that the sufficient adjustment set of covariates is age, gender, and BMI. CFS-like illness is abbreviated as CFS in this graph.

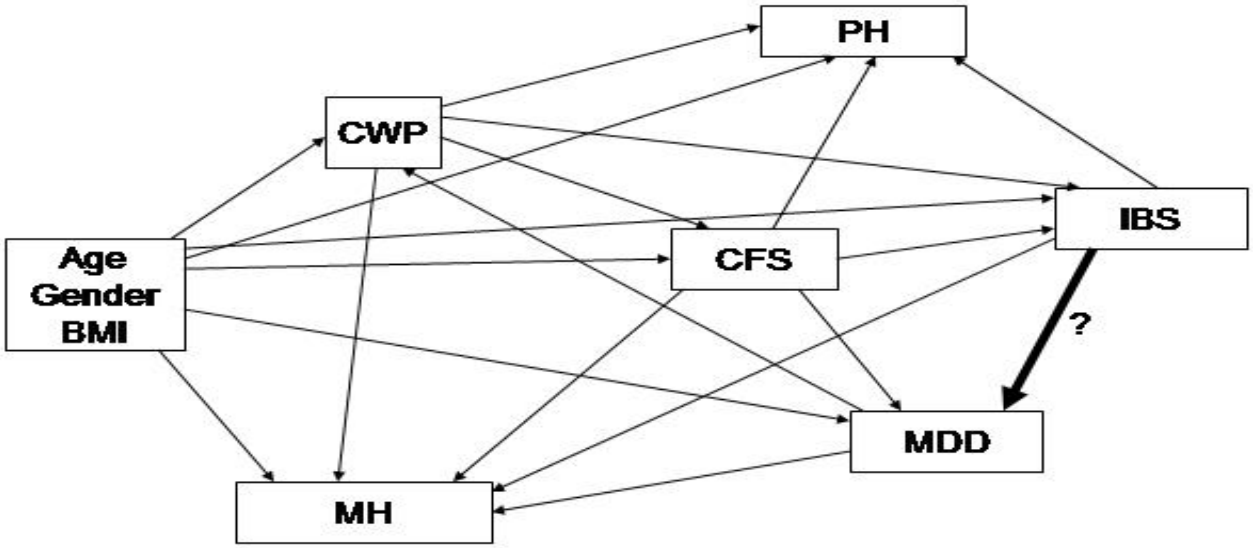
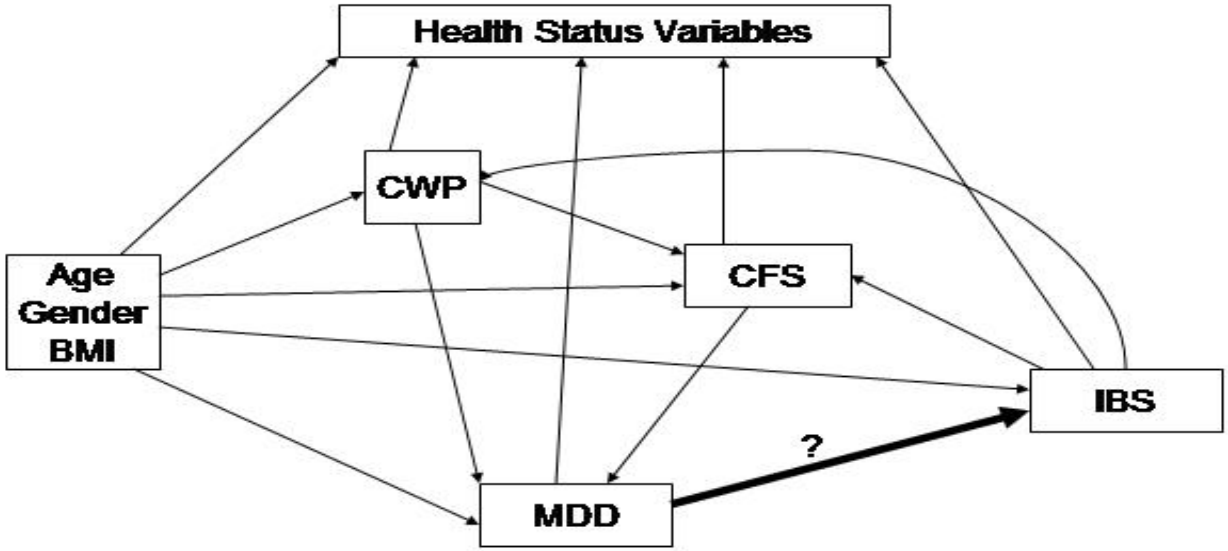


Figure 2.8: Directed acyclic graph to assess associations between MDD and IBS in the SALT study, 2002. Analysis of this graph implies that the sufficient adjustment set of covariates is age, gender, and BMI. CF-like illness is abbreviated as CFS in this graph. The three self-rated health measures are included in as Health Status Variables in this graph.



2.6 REFERENCES

1. Rome II: the functional gastrointestinal disorders: diagnosis, pathophysiology, and treatment: a multinational consensus. 2nd ed. McLean, VA: Degnon Associates; 2000.

2. Taub E, Cuevas JL, Cook EW, 3rd, Crowell M, Whitehead WE. Irritable bowel syndrome defined by factor analysis. Gender and race comparisons. Dig Dis Sci 1995;40(12):2647-55.

3. Whitehead WE, Crowell MD, Bosmajian L, Zonderman A, Costa PT, Jr., Benjamin C, et al. Existence of irritable bowel syndrome supported by factor analysis of symptoms in two community samples. Gastroenterology 1990;98(2):336-40.

4. Talley NJ, Holtmann G, Agreus L, Jones M. Gastrointestinal symptoms and subjects cluster into distinct upper and lower groupings in the community: a four nations study. Am J Gastroenterol 2000;95(6):1439-47.

5. Vanner SJ, Depew WT, Paterson WG, DaCosta LR, Groll AG, Simon JB, et al. Predictive value of the Rome criteria for diagnosing the irritable bowel syndrome. Am J Gastroenterol 1999;94(10):2912-7.

6. Whitehead W, Palsson O, Levy R, Feld A, VonKorff M, Drossman D, et al. Agreement of Rome Criteria with Clinical Diagnosis of Irritable Bowel (IBS). In: RS S, editor.; 2003. p. Email correspondence of abstract.

7. Leibbrand R, Cuntz U, Hiller W. Assessment of functional gastrointestinal disorders using the Gastro-Questionnaire. Int J Behav Med 2002;9(2):155-72.

8. Romero Y, Thistle JL, Longstreth GF, Harmsen WS, Schleck CD, Zinsmeister AR, et al. A questionnaire for the assessment of biliary symptoms. Am J Gastroenterol 2003;98(5):1042-51.

9. Steiner JL, Tebes JK, Sledge WH, Walker ML. A comparison of the structured clinical interview for DSM-III-R and clinical diagnoses. J Nerv Ment Dis 1995;183(6):365-9.

10. Riskind JH, Beck AT, Berchick RJ, Brown G, Steer RA. Reliability of DSM-III diagnoses for major depression and generalized anxiety disorder using the structured clinical interview for DSM-III. Arch Gen Psychiatry 1987;44(9):817-20.

11. Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry 1992;49(8):624-9.

12. Skre I, Onstad S, Torgersen S, Kringlen E. High interrater reliability for the Structured Clinical Interview for DSM-III-R Axis I (SCID-I). Acta Psychiatr Scand 1991;84(2):167-73.

13. Segal DL, Hersen M, Van Hasselt VB. Reliability of the Structured Clinical Interview for DSM-III-R: an evaluative review. Compr Psychiatry 1994;35(4):316-27.

14. Williams JB, Gibbon M, First MB, Spitzer RL, Davies M, Borus J, et al. The Structured Clinical Interview for DSM-III-R (SCID). II. Multisite test-retest reliability. Arch Gen Psychiatry 1992;49(8):630-6.

15. Hodgins DC, Dufour M, Armstrong S. The reliability and validity of the inventory to diagnose depression in alcohol-dependent men and women. J Subst Abuse 2000;11(4):369-78.

16. Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 2003;60(9):929-37.

17. Zimmerman M, Coryell W, Corenthal C, Wilson S. A self-report scale to diagnose major depressive disorder. Arch Gen Psychiatry 1986;43(11):1076-81.

18. Uehara T, Sato T, Sakado K, Kameda K. Discriminant validity of the Inventory to Diagnose Depression between patients with major depression and pure anxiety disorders. Psychiatry Res 1997;71(1):57-61.

19. Terluin B, van Hout HP, van Marwijk HW, Ader HJ, van der Meer K, de Haan M, et al. Reliability and validity of the assessment of depression in general practice: the Short Depression Interview (SDI). Gen Hosp Psychiatry 2002;24(6):396-405.

20. Sheeran T, Zimmerman M. Case identification of depression with self-report questionnaires. Psychiatry Res 2002;109(1):51-9.

21. Tondo L, Burrai C, Scamonatti L, Weissenburger J, Rush J. Comparison between clinician-rated and self-reported depressive symptoms in Italian psychiatric patients. Neuropsychobiology 1988;19(1):1-5.

22. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. The lifetime history of major depression in women. Reliability of diagnosis and heritability. Arch Gen Psychiatry 1993;50(11):863-70.

23. Kendler KS, Pedersen NL, Neale MC, Mathe AA. A pilot Swedish twin study of affective illness including hospital- and population-ascertained subsamples: results of model fitting. Behav Genet 1995;25(3):217-32.

24. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34(3):220-33.

25. Ruta DA, Abdalla MI, Garratt AM, Coutts A, Russell IT. SF 36 health survey questionnaire: I. Reliability in two patient based studies. Qual Health Care 1994;3(4):180-5.

26. Roberts R, Hemingway H, Marmot M. Psychometric and clinical validity of the SF-36 General Health Survey in the Whitehall II study. British Journal of Health Psychology 1997;2:285-300.

27. Jenkinson C, Wright L, Coulter A. Criterion validity and reliability of the SF-36 in a population sample. Qual Life Res 1994;3(1):7-12.

28. Mid-Atlantic Twin Registry, Medical College of Virginia and Virginia Commonwealth University. In. Richmond, VA.

29. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, D.C.: American Psychiatric Association; 1994.

30. The Swedish Twin Registry. In. Stockholm, Sweden: Karolinska Institutet.

31. Pedersen NL, Lichtenstein P, Svedberg P. The Swedish Twin Registry in the third millennium. Twin Res 2002;5(5):427-32.

32. Lichtenstein P, De Faire U, Floderus B, Svartengren M, Svedberg P, Pedersen NL. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. J Intern Med 2002;252(3):184-205.

33. Svedberg P, Johansson S, Wallander MA, Hamelin B, Pedersen NL. Extra-intestinal manifestations associated with irritable bowel syndrome: a twin study. Aliment Pharmacol Ther 2002;16(5):975-83.

34. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51(1):8-19.

35. Institute S. SAS. In. 8.02 ed. Cary, NC: SAS Institute Inc.; 1999-2001.

36. Rothman K. Epidemiology: An Introduction. New York, NY: Oxford University Press; 2002.

37. Kleinbaum D, Kupper L, Muller K, Nizam A. Applied Regression Analysis and Other Multivariable Methods. Third Edition ed. Pacific Grove, CA: Duxbury Press; 1998.

38. Stokes ME, Davis CS, Koch GG. Categorical Data Analysis Using the SAS System. 2nd Edition ed. Cary, NC: SAS Institute Inc.; 2000.

39. Kleinbaum D. Logistic Regression: A self-learning text. New York, NY: Springer-Verlag; 1994.

40. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol 2002;155(2):176-84.

41. Greenland S, Brumback B. An overview of relations among causal modelling methods. Int J Epidemiol 2002;31(5):1030-7.

42. Robins JM. Data, design, and background knowledge in etiologic inference. Epidemiology 2001;12(3):313-20.

43. Poole C. Low P-values or narrow confidence intervals: which are more durable? Epidemiology 2001;12(3):291-4.

44. Rothman KJ, Greenland S. Modern Epidemiology. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998.

45. Rothman K. Episheet: Spreadsheets for the analysis of epidemiologic data. In. February 21, 2002 ed; 2001.

3 RESULTS MANUSCRIPT 1. ASSOCIATIONS WITH ROME II IBS IN A US TWIN REGISTRY

3.1 ABSTRACT

Objective. To investigate the prevalence and associations of self-reported lifetime Rome II IBS in a U.S. twin registry.

Methods. We performed a nested case-control study on a subset of the population-based Mid-Atlantic Twin Registry (MATR). Variables representing Rome II IBS, co-morbid disorders, self-rated quality of life (SF-12), and personal characteristics were obtained by mailed questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were used as measures of association between IBS and each covariate, using generalized estimating equations to account for the non-independence of twin pairs.

Results. 4,307 participants responded with complete covariate information. 4.7% (95% CI: 4.1, 5.4) fulfilled the Rome II IBS symptom criteria. Female gender and several co-morbid disorders were positively associated with IBS. Age, body mass index, and SF-12 scores demonstrated approximately null associations with IBS.

Conclusion. The lifetime prevalence of IBS in this study was similar to prior research using the Rome II criteria, but lower than in prior studies employing different symptom criteria for IBS, as expected due to the greater specificity of the Rome II criteria. Rome II IBS criteria were associated with similar IBS risk factors as prior IBS criteria. Additionally, in this general population, we noted that those with major depressive disorder are twice as likely to satisfy Rome II IBS symptom criteria. More research into the possible common etiology of these two disorders is warranted.

3.2 INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic disorder of the gastrointestinal (GI) tract. It is characterized by exacerbations and remissions of abdominal pain and/or discomfort, leading to alterations in bowel habits (1-5). The diagnosis of IBS is based on symptom criteria and exclusion of structural disease (1, 3). Community-based studies of IBS have estimated the prevalence to range from 9-22%, depending on the symptom criteria employed by the study, Table 3.1 (4, 6-13). Upon closer inspection of these studies, the majority did not employ the most recent adaptation of the symptom criteria, the Rome II criteria (14), but either the Manning or Rome I criteria, from which the Rome II criteria evolved. Additionally, most studies were not performed in the United States. A subset of these studies also presented IBS prevalence by gender, Table 3.2 (13, 15-17).

The Rome II criteria for IBS are preferred over prior criteria because they are readily applied and identify a core group of individuals that exhibit the typical waxing and waning of IBS symptoms (4). Moreover, the use of one standardized criteria across studies would allow for more accurate cross study comparisons and may, in the long term, lead to a better understanding of the pathogenesis of IBS.

Although there has been little research regarding risk factors associated with Rome II IBS, the association between Manning and Rome I IBS and major depressive disorder (MDD) has been of great interest. MDD and IBS have demonstrated a co-occurrence of about 50%, which is much higher than the expected co-occurrence of IBS and MDD. Assuming that IBS and MDD are independent, and varying their prevalence rates between 5 and 20% for IBS and 15-20% for MDD, one would expect a co-occurrence range between 1-4% in the community (18-24). However the majority of studies noting a higher than expected co-occurrence were not performed in the community (18, 24-26) and used earlier symptom criteria for IBS.

Notably, community-based studies had a co-occurrence of 25% (27). This difference between community and non-community studies could be explained by treatment seeking bias because those individuals that actively seek treatment have self-selected themselves to see a physician and thus may be different than those not seeking care. Specifically, individuals

seeking care for IBS-related symptoms document a more depressed psychopathology than individuals not seeking care (18, 21). Thus non-community populations are a biased sample when examining the co-occurrence of MDD and IBS.

This study addresses some of the shortcomings of prior IBS research. It employs the most recent symptom criteria, Rome II, and is population-based to eliminate any possible effects of treatment-seeking bias. We aim to estimate the population prevalence of Rome II IBS, to assess Rome II-defined IBS associations with MDD, other co-morbid disorders, and other demographic variables in order to more fully understand the population of individuals that fulfill the Rome II IBS symptom criteria.

3.3 METHODS

3.3.1 Study Population

We conducted a nested case-control study on a subset of the Mid-Atlantic Twin Registry (MATR) (N=4,591). The MATR cohort is a population-based registry of twin pairs ascertained from birth and school system records in Virginia, North Carolina, and South Carolina (28). The study was reviewed and approved by the Institutional Review Boards (IRB) at Virginia Commonwealth University, the for-profit Western IRB, the University of Washington at Seattle, and the University of North Carolina at Chapel Hill. All subjects provided written informed consent.

3.3.2 Data Collection

During the last quarter of 1999, a detailed survey was mailed to approximately 15,000 individual participants in the MATR. Of these, 4,591 returned completed surveys for a response rate of 31%. After the initial mailing, the study was halted by the US Department of Health and Human Services Office for Human Research Protections (29, 30), as was all ongoing human subjects research at Virginia Commonwealth University. This shutdown was for reasons not directly related to this study. However the shutdown led to the early termination of this study, and the planned repeat mailings, non-responder telephone follow-up, and clinical evaluations that were planned were not conducted, see Furberg et al. for more discussion (31). Additionally zygosity data were available on only a minority of the subjects.

3.3.3 Irritable Bowel Syndrome (IBS) Assessment

Subjects completed a questionnaire that captured lifetime history of IBS according to the Rome II criteria (14). Briefly, these criteria were operationalized as the lifetime occurrence of pain or discomfort in their lower abdomen for ≥ 3 months in the past year, which need not be consecutive, along with at least two of the following for a duration of 3 months: bowel movement relieving the pain or discomfort; change in number of bowel movements (either more or less than usual); or a change in the consistency of

their stool (either harder or looser than usual). Individuals who reported a personal history of ulcerative colitis (n=12), Crohn's disease (n=9), or both (n=3) were excluded.

3.3.4 Covariate Assessment

The mailed survey also used previously validated instruments to assess symptomatology consistent with a lifetime history of major depressive disorder (MDD), chronic widespread pain (CWP), and chronic fatigue syndrome (CFS)-like illness. Assessment of lifetime MDD was based on a questionnaire adaptation (31-33) of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), criteria (34). The presence of lifetime CWP assessment used an adaptation of the American College of Rheumatology criteria for fibromyalgia (35, 36). Lifetime presence of CFS-like illness was assessed using a module similar to the US Center for Disease Control (CDC) consensus criteria (37) for chronic fatigue syndrome (38). No clinical examinations were conducted.

Participants also answered questions on self-rated health, and current height and weight at the time of the questionnaire. The self-rated health questions comprised the SF-12 (39) and were divided into mental and physical health subscales for analysis. The SF-12 assesses health-related quality of life with scores ranging from 0 to 100 where a higher score is

indicative of better health-related quality of life. Current height and weight were used to calculate current body mass index (BMI, kg/m^2).

3.3.5 Statistical Analyses

Odds ratios (OR) with 95% confidence intervals (CIs) were used as measures of association between IBS and each covariate. To account for the inherent relatedness of twin pairs, we used generalized estimating equations (40). All analyses were conducted using the SAS software (41). Additionally, analyses were completed for both sexes combined and stratified by sex, due to the higher prevalence of IBS among women. However, as gender stratified associations differed little from the overall pattern of associations in the combined data (data not shown), only the results from the complete sample will be reported.

The distribution of covariates in the study population, stratified by IBS case-control status, was assessed to determine similarity between the case and control populations. This allowed for an assessment of whether the demonstrated association may be due to differences in the distributions. We used generalized estimating equations with linear and logistic regression models. Continuous covariates were analyzed using appropriate categories for this part of the analysis. For dichotomous and categorical covariates, frequencies are presented. χ^2 statistics were calculated to determine if missing covariate information varied by IBS status (results not shown),

thereby allowing us to assess if missing information was associated with IBS case status.

Multivariable Models. Eight multivariable models, one for the association between each covariate of interest and IBS, were constructed using a backward elimination procedure. For each model, effect measure modification was assessed before confounding. At the end of the confounding assessment, the final fully adjusted multivariable model assessing the association between the covariate of interest in that model and IBS in this population was obtained. Thus a multivariable model was obtained using each covariate (age at interview, gender, BMI, MDD, CWP, CFS-like illness, SF-12 mental health score, and SF-12 physical health score) as the main exposure in one of the eight models.

Covariate Selection. For each model potential covariates and confounders were identified by analyzing directed acyclic graphs (42). A directed acyclic graph was drawn and analyzed for each exposure based on a review of the literature to help establish potential covariates as confounders. Potential covariates that were affected by the exposure or affected by the outcome were not considered as potential confounders or included as covariates in any models.

Effect Measure Modification. In each model, we assessed effect modification of the association between the main exposure in that model and

IBS using logistic regression models (employing generalized estimating equations) in which first-order interaction terms between the exposure for that model and each of the potential confounders for that association were created. To determine if any potential interactions were significant effect modifiers, we compared results from two logistic regression models, one with all the potential interactions and one without any interaction terms, both containing the main exposure and all linear terms of the interactions. This allowed for a 'chunk' test using the likelihood ratio test and its associated χ^2 statistic to determine if the interaction terms as a whole could be eliminated from the model (43). If the χ^2 statistic associated with this test was not significant at $p<0.20$, the removed interaction terms were not significant to the exposure and IBS association and thus remained eliminated from the model (43). However, if the χ^2 statistic associated with this 'chunk' test for interaction was significant at $p<0.20$, at least some or all of the interaction terms were significant. Thus all the interaction terms were returned to the model for further assessment using backward elimination to examine each interaction term one at a time in order to eliminate insignificant variables from the model (43). Once all potential effect modifiers were assessed, covariates involved in significant effect modification terms were included as linear terms in all remaining logistic models.

Confounding. Once the assessment of effect measure modification was complete, we assessed confounding for each model. To determine if a

potential confounder of the exposure and IBS association did confound the association in our population, logistic regression models (using generalized estimating equations) with and without the potential confounder were compared using the OR for the main exposure. First, we obtained results for the model with the exposure, all potential confounders, and any significant interaction terms (full model). Using these results, the potential confounder not involved in any interaction terms with the largest associated p-value was removed from the full model and we obtained modeling results for this adjusted model. In order to determine if the inclusion of this term confounded the association, the OR from this adjusted model was compared to the OR from the full model. If $\ln|(OR_{full}/OR_{adjusted})| > 0.10$, then the variable was a confounder and was returned to the full model; otherwise the variable was not a confounder of this association and was eliminated from the model (43). This process was repeated for all potential confounders, where the full model changed based on the results of the prior confounder assessment (i.e. if the potential confounder did not confound the association, then that model became the full model), until all potential confounders were assessed.

Sensitivity Analysis. Since the order of onset of IBS and MDD is not known, we chose IBS as our dependent variable and assumed that MDD preceded IBS. In an effort to determine if this assumption affected the association between IBS and MDD, we performed a sensitivity analysis in which MDD was the dependent variable and IBS was the primary exposure.

3.4 RESULTS

3.4.1 Sample Description

Of all individual twins who responded (N=4,591), 4,307 individuals had complete covariate information and were used in this analysis. Among this sample, 203 participants (4.7%, 95% CI: 4.1, 5.4; women 5.7%, 95% CI: 4.9, 6.6; men 2.9%, 95% CI: 2.1, 3.8) fulfilled the Rome II IBS criteria. Due to the higher prevalence among women of IBS, and also among most other covariates of interest, the associations between IBS and each covariate were examined by gender; however, no gender interactions were noted and thus all subsequent results are presented for males and females combined. Based on the χ^2 statistics (results not shown), missing data for the variables we examined did not differ by IBS status.

3.4.2 Descriptive Statistics

Irrespective of IBS case-control status, the gender distribution of the sample had a female predominance (67.9%). Characteristics of the sample population, stratified by IBS case-control status are shown in Table 3.3. No major differences in BMI or age between cases and controls were noted. The co-morbid disorders, CWP, CFS-like illness, and MDD, were more common in cases than in controls. Moreover, a higher percentage of controls had above average mental and physical health scores on the SF-12 than cases.

3.4.3 Multivariable Analyses

Effect Measure Modification Assessment. For each of the eight models, the results of the likelihood ratio test determined that no effect measure modification was identified when assessing the association between each exposure (MDD, gender, BMI, CFS-like illness, CWP, age, mental health score, and physical health score) and IBS.

Confounding Assessment. Since no effect modifiers were identified, each model assessed the a priori identified potential confounders (determined based on the directed acyclic graph analysis) as true confounders in our population. The multivariable models for the associations between BMI and IBS, and CFS-like illness and IBS were adjusted for gender and familial clustering of twins. The multivariable models for the other exposures were only adjusted for the familial clustering of twins.

Summary of Multivariable Models. For the multivariable models, female gender, MDD, CWP, and CFS-like illness were positively associated with IBS (Table 3.4). Age at interview and BMI were not associated with IBS. The mental and physical self-rated health questions demonstrated inverse associations with IBS.

Sensitivity Analysis. To assess if the timing of onset between MDD and IBS had an effect on their association, we performed a sensitivity analysis. In this analysis, we found little difference (2.5%) in the magnitude of

the association, with overlapping confidence intervals and good precision (Table 3.5). Thus, the choice of dependent variable did not affect any association in this analysis, and we were unable to make any inferences about the timing of the onset of these disorders. However, it should be noted that most individuals (54%) who fulfilled both the lifetime IBS and lifetime MDD (N=69) symptomatology also stated that the onset of MDD occurred before the onset of IBS (data not shown).

3.5 DISCUSSION

In this subset of the Mid-Atlantic Twin Registry, the lifetime prevalence of Rome II IBS was 4.7%. This finding is supported by previous research which reported a lower IBS prevalence when using Rome II symptom criteria in place of earlier symptom criteria (4, 8, 11, 12, 17, 44). Similar to previous studies, women were twice as likely to satisfy IBS criteria (13, 15-17). In our study, a perception of good physical and mental health were associated with a decrease in the odds of lifetime IBS, whereas chronic fatigue syndrome-like illness and chronic widespread pain were associated with an increase in the odds of IBS. BMI and age were not associated with IBS. MDD and IBS were positively associated with IBS, where those with MDD were twice as likely to satisfy the Rome II IBS symptom criteria.

Prior studies using Rome II IBS criteria (Table 3.1) have demonstrated 1-year IBS prevalences between 3.3 and 7% (4, 8, 11, 12, 17, 44). Earlier

IBS symptom criteria (Manning and Rome I, Table 3.1) have demonstrated a higher population prevalence of IBS, between 9-22% (4, 5, 45). Thus while our lifetime prevalence of 4.7% is lower than that of prior studies which employed earlier symptom criteria, it is consistent with results of prior studies using the more recent Rome II symptom criteria.

Women were twice as likely as men to satisfy the symptom criteria for IBS in our study. This is consistent with other population-based studies in North America that demonstrated a 2:1 predominance of women meeting IBS symptom criteria (15, 46). Additionally, studies performed in Bangladesh (13) and China (17) also reported a higher prevalence of IBS in women in comparison to men. However, in studies performed since 2003 (Table 3.2), the 2:1 predominance of women satisfying IBS symptom criteria is not upheld. In these studies, the ratio of female:male IBS prevalence was clustered between 1.2 and 1.5, regardless of which IBS symptom criteria were used. When looking solely at the Rome II IBS symptom criteria, the ratio spans from 1 to 2 in these studies. Thus our finding of a female predominance of IBS using the Rome II criteria is consistent with earlier research, and with the results of Sperber et al. (47) which was performed in Israel and used the Rome II IBS symptom criteria. However while the magnitude of this ratio conflicts with the more recent IBS studies in Table 3.2, women in general have a higher prevalence of IBS, regardless of which IBS criteria (Manning, Rome I, or Rome II) were applied. Thus, our research supports the notion of

women satisfying IBS criteria more often than men, and further demonstrates the robustness of gender being associated with IBS, since its association with IBS is independent of the symptom criteria employed.

This study demonstrated an association between MDD and IBS that was precise (OR 2.0, 95%CI 1.5, 2.7; p<0.0001). Thus individuals with MDD were twice as likely to satisfy lifetime IBS criteria. This estimate is similar to that of Talley et al. (11), where an OR of 2.0 (95% CI: 0.7, 5.6) was documented when comparing Rome criteria for IBS to controls. Two other studies also demonstrated higher odds ratios: 3.5 (95% CI: 3.4, 3.6) (23) and 14.3 (95% CI: 13.2, 15.4) (24) both using prior IBS criteria. Additional research into the association between MDD and IBS has demonstrated that approximately half of individuals fulfilling criteria for one disorder also meet symptom criteria for the other disorder (20, 22, 24). In our study, the overlap of the two disorders was slightly less, as 32% of individuals meeting Rome II IBS symptom criteria also fulfilled criteria for MDD. This could be explained because our study was population-based, whereas most prior research examining IBS and MDD used individuals who were actively seeking care for either one or both disorders. Use of such a treatment-seeking population can be biased, and thus may have a higher percent overlap between IBS and MDD due to individuals assessing doctors more often and thus having more opportunities for a diagnosis of one or both disorders. Thus our results are

consistent with most prior research demonstrating an association between MDD and IBS.

The timing of onset of IBS in comparison to MDD has been an issue in this study and in previous studies (7, 20, 21, 24, 48). It is still not known whether the onset of IBS precedes that of MDD or if the onset of MDD precedes that of IBS. Our study was cross-sectional, and as such, we chose which disorder to be the dependent variable for the analyses, thus assuming that the onset of the independent variable event (MDD) occurred before the onset of the dependent variable event (IBS). Our sensitivity analysis attempted to determine if this choice of the timing of onset between these disorders affected any association. The results demonstrated no difference in the association had MDD been the dependent variable among this sample. Additionally, the number of individuals in our study that suffered from both disorders was too small for a further examination of this question, and there is clearly more work to be done to establish the temporality question, ideally in the context of a prospective cohort study.

Individuals satisfying the Rome II IBS symptom criteria had lower mean SF-12 scores for both the mental and physical health components than those not satisfying the IBS symptom criteria in our study. This finding has also been documented in prior studies using both the longer SF-36 (49-52) as well as in the SF-12 (53). In population-based studies, individuals with IBS

also had lower SF-36 scores (54) in comparison to healthy population controls. Our finding is consistent with prior studies, and suggests an association between IBS and worse health-related quality of life, regardless of the IBS definition used.

In our study, chronic fatigue syndrome-like illness and chronic widespread pain (CWP) were associated with an increased odds of lifetime IBS. Previous studies of CFS have demonstrated an association with IBS (55-57), where CFS patients were more likely to demonstrate symptoms of IBS than healthy controls or non-fatigued co-twins. Moreover, the prevalence of IBS among those with CFS was between 50-92% (55, 57, 58), which is higher than in our study, where the prevalence of IBS was 18% among those with CFS-like illness. Although the prevalence of IBS in these studies differs in magnitude, the effect estimates from our study are in the same direction and for at least one twin study which found fatigued twins 4-10 times more likely to be diagnosed with IBS compared to non-fatigued twins, our 95% confidence interval encompasses its lower bound (57).

Previous studies have also documented a co-occurrence between CWP and IBS, at a rate of 32-66% (59-62). Additionally, in a small study of 33 women with CWP, 39% had a current diagnosis of IBS and 52% had a lifetime diagnosis of IBS (63). Thus our findings of a higher prevalence of IBS among those with CFS-like illness or CWP are generally consistent with the

previous literature, although the actual magnitude of the association varies across studies.

We found no evidence of age or BMI influencing the risk of IBS. Prior studies of age and IBS have found that most individuals fulfilling symptom criteria for IBS have symptoms by age 35 (3), and that individuals younger than 45 were more likely to be diagnosed with IBS than those older than 45 (15). However, other studies have shown that the prevalence of IBS varies minimally with age (64). Thus in prior studies age was not a risk factor for IBS, but aided in describing the population that fulfills the IBS criteria, which is consistent with our findings. Prior research on the relationship between BMI and IBS has been conflicting. Most studies have found no association between BMI and IBS (65-68). However, a more recent community-based study in Croatia demonstrated that for every increase in BMI of 5 kg/m^2 an increased risk of IBS of 36% was reported (69). In contrast, our study found no association between BMI and IBS, which is consistent with the majority of studies.

While this study increases the knowledge base of Rome II IBS, there are several limitations to consider. First, the early termination of this study did not allow for the follow-up mailings, which may have substantially increased the response rate (see Furberg et al. for a more detailed discussion) (31). Thus the sample may be biased due to only obtaining information on those

who were early responders, those who did not move, and those with correct addresses. Additionally, the information contained in this study is all self-report. No physical exams, psychiatric evaluation, or medical record reviews were performed, which could lead to misclassification for some disorders. Lastly, there was limited information on the timing of onset of MDD and IBS. As such, we made a choice as to which disorder preceded the onset of the other disorder, which could be incorrect.

Despite these limitations, there are several strengths of this study. First, our study used data from a large population-based study. Therefore, the effect of treatment-seeking bias should have been kept to a minimum. Additionally, the use of a population-based sample should include individuals of all levels of disease severity. Second, the Rome II symptom criteria for IBS were employed, which are arguably superior to prior criteria (70). Thus, this study allows for a better understanding of the Rome II criteria and its risk factors in comparison to prior studies which applied earlier symptom criteria. Lastly, we performed a sensitivity analysis in an attempt to address the timing of onset of IBS and MDD. The results indicated that in our study, timing of onset did not mask any association, however much work remains to be done to establish the timing of onset of IBS and MDD.

In conclusion, we used a population-based study to assess previously known IBS associations, using the most recent IBS criteria, the Rome II

criteria. Results demonstrate that IBS associations using previous IBS criteria are similar to our results using the Rome II criteria. Additionally, our study adds to the growing body of literature (7, 15, 18, 24) that demonstrates individuals with IBS being more likely to have MDD than those without IBS (Table 3.3: 32% versus 19%). While these results support prior research, they are also unique due to the use of the most recent IBS criteria, Rome II, and the use of a population-based study. Many prior studies examining disorders co-morbid with IBS have used populations of individuals seeking care (7, 15, 18, 20), and thus may suffer from treatment seeking bias and are not representative of all individuals with IBS. Since an individual's quality of life is associated with IBS and other co-morbid disorders, reasons for the large overlap among individuals with IBS and chronic widespread pain, chronic fatigue syndrome-like illness, and major depressive disorder should receive increased research in order to investigate the possibility of a common, as yet unknown, etiology in an effort to alleviate the burden of these disorders in the population and on health care resources.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES

Table 3.1. Prior community-based studies estimating IBS[1] prevalence.

Study	Sample Size	IBS criteria	Prevalence (95% CI[1])	Country
Boyce et al. (4)	3,235	Manning	13.6 (12.2, 15.1)	Australia
		Rome I	4.4 (3.6, 5.2)	
		Rome II	6.9 (5.9, 8.0)	
Hu et al. (7)	1,649	Rome I	4.1 (3.2, 5.1)	China
Kwan et al. (12)	1,000	Rome II	6.6 (5.1, 8.2)	Hong Kong
Masud et al. (13)	2,632	Rome I	8.5 (7.5, 9.6)	Bangladesh
Mearin et al. (8)	1,932	Manning	10.3 (9.0, 11.7)	Spain
		Rome I	12.1 (10.7, 13.6)	
		Rome II	3.3 (2.5, 4.1)	
Saito et al. (9)	892	Manning (≥ 2)	20.4 (16.7, 24.2)	US
		Rome I	12.1 (10.0, 16.2)	
		Rome II	8.5 (5.9, 11.1)	
Talley et al. (10)	730	Manning	13 (11, 16)	Australia
		Rome I	12 (10, 15)	
Talley et al. (6)	3,022	Manning	18 (16.7, 19.4)	US
Talley et al. (11)	890	Manning	12.7 (10.6, 15)	New Zealand
		Rome II	4.3 (3.1, 5.7)	
Thompson et al. (46)	1,149	Rome I	13.5 (11.6, 15.5)	Canada
		Rome II (modified)	12.1 (10.3, 14.1)	
Lau et al. (17)	1,278	Rome II	3.2 (2.3, 4.2)	Hong Kong
Hungin et al. (71)	5,009	All	14.1 (11.6, 16.8)	US
		Rome II	6.7 (4.3, 9.6)	
Yilmaz et al. (72)	3,000	Rome II	10.2 (9.1, 11.3)	Turkey
Sperber et al. (47)	981	Rome II	2.9 (1.9, 4.1)	Israel
Dapoigny et al. (73)	15,106	Rome II	4.7 (4.4, 5.0)	France
Gwee et al. (74)	2,276	Manning (>1)	11.0 (9.7, 12.3)	Singapore
		Rome I	10.4 (9.1, 11.6)	
		Rome II	8.6 (7.5, 9.8)	
Xiong et al. (75)	4,178	Manning	11.5 (10.6, 12.5)	South China
		Rome II	5.7 (4.9, 6.4)	
Kanazawa et al. (76)	417	Rome II	14.2 (11.0, 17.7)	Japan
Talley et al. (68)	923	Manning	18.2 (15.8, 20.7)	New Zealand
Celebi et al. (77)	1,766	Rome II	6.3 (5.2, 7.5)	Turkey
Hoseini-Asl et al. (78)	4,762	Rome II	5.8 (5.2, 6.5)	Iran
Hillila et al. (79)	3,631	Manning (>2)	16.2 (15.0, 17.4)	Finland
		Manning (>3)	9.7 (8.8, 10.7)	
		Rome I	5.5 (4.8, 6.3)	
		Rome II	5.1 (4.4, 5.8)	
Saito et al. (80)	643	Rome II	4.7 (3.2, 6.5)	US

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime. CI=confidence interval.

Table 3.*2.* Gender stratified IBS[1] prevalence from prior studies.

Study	IBS criteria	Male sample size	Male prevalence (95% CI[1])	Female sample size	Female prevalence (95% CI)
Saito et al. (80)	Rome II	306	4.9 (2.7, 7.6)	337	4.5 (2.5, 7.0)
Hillila et al. (79)	Manning (>2)	1,980	13.1 (11.4, 14.8)	1,668	19.2 (17.4, 20.9)
	Manning (>3)		8.3 (7.0, 9.7)		11.2 (9.8, 12.6)
	Rome I		5.1 (4.0, 6.2)		6.1 (5.0, 7.2)
	Rome II		5.1 (4.0, 6.2)		5.3 (4.3, 6.3)
Gwee et al. (74)	Manning (>1)	1,143	9.5 (7.8, 11.3)	1,133	12.6 (10.7, 14.6)
	Rome I		9.0 (7.4, 10.8)		11.7 (9.9, 13.6)
	Rome II		7.8 (6.3, 9.5)		9.4 (7.8, 11.3)
Xiong et al. (75)	Manning	1,907	9.7 (8.5, 10.9)	2,271	12.95 (11.5, 14.5)
	Rome II		5.03 (4.2, 6.0)		6.3 (5.3, 7.4)
Kanazawa et al. (76)	Rome II	203	12.9 (8.7, 17.7)	214	15.5 (10.8, 20.8)
Talley et al. (68)	Manning	445	14.6 (11.6, 17.9)	484	22.3 (18.6, 26.3)
Celebi et al. (77)	Rome II	802	5.0 (3.6, 6.6)	964	7.4 (5.8, 9.1)
Yilmaz et al. (72)	Rome II	1,479	8.0 (6.7, 9.4)	1,521	12.4 (10.8, 14.1)
Sperber et al. (47)	Rome II	441	1.8 (0.7, 3.3)	540	3.7 (2.3, 5.5)

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime. CI=confidence interval.

Table 3.3. Descriptive statistics of 203 IBS cases and 4,104 controls in the MATR Study[1].

Characteristic	Cases, n (%) (n=203)	Controls, n (%) (n=4,104)	OR[1]	95% CI[1]
Gender				
Males	44 (21.7)	1,478 (36.0)	1.0	Referent
Females	159 (78.3)	2,626 (64.0)	2.03	1.44, 2.85
Major Depressive Disorder	64 (31.5)	762 (18.6)	2.00	1.48, 2.71
Chronic Widespread Pain	47 (23.2)	294 (7.2)	3.89	2.74, 5.53
Chronic Fatigue Syndrome-like Illness	29 (14.3)	129 (3.1)	5.13	3.30, 7.97
Body Mass Index				
≤ 24.9 kg/m^2	75 (36.9)	1,744 (42.5)	1.0	Referent
25-29.9 kg/m^2	70 (34.5)	1,393 (33.9)	1.17	0.83, 1.64
≥30 kg/m^2	58 (28.6)	967 (23.6)	1.40	0.98, 2.00
Age at Interview			1.00	0.98, 1.01
< 36	49 (24.1)	1,016 (24.8)		
36-43	57 (28.1)	1,080 (26.3)		
44-51	54 (26.6)	1,091 (26.6)		
> 51	43 (21.2)	917 (22.3)		
Mental Health Score			0.97	0.95, 0.98
< 50	98 (48.3)	1,266 (30.8)		
≥ 50	105 (51.7)	2,838 (69.2)		
Physical Health Score			0.95	0.94, 0.97
< 50	103 (50.7)	997 (24.3)		
≥ 50	100 (49.3)	3,107 (75.7)		

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime (past three months). OR=odds ratio. CI=confidence interval.

Table 3.4. Assessment of effect modification and confounding. Adjusted odds ratios (ORs) and 95% CIs for IBS in relation to descriptive characteristics, co-morbid disorders, and self-rated health.

Characteristic	Cases, n (%) (n=203)	Controls, n (%) (n=4,104)	OR[1,2]	95% CI[1]
Gender				
Males	44 (21.7)	1,478 (36.0)	1.0	Referent
Females	159 (78.3)	2,626 (64.0)	2.03	1.44, 2.85
Major Depressive Disorder	64 (31.5)	762 (18.6)	2.00	1.48, 2.71
Chronic Widespread Pain	47 (23.2)	294 (7.2)	3.89	2.74, 5.53
Chronic Fatigue Syndrome-like Illness	29 (14.3)	129 (3.1)	4.67†	3.01, 7.26
Body Mass Index				
≤ 24.9 kg/m^2	75 (36.9)	1,744 (42.5)	1.0	Referent
25-29.9 kg/m^2	70 (34.5)	1,393 (33.9)	1.34†	0.95, 1.90
≥30 kg/m^2	58 (28.6)	967 (23.6)	1.48†	1.04, 2.11
Age at Interview			1.00	0.98, 1.01
< 36	49 (24.1)	1,016 (24.8)		
36-43	57 (28.1)	1,080 (26.3)		
44-51	54 (26.6)	1,091 (26.6)		
> 51	43 (21.2)	917 (22.3)		
Mental Health Score			0.97	0.95, 0.98
< 50	98 (48.3)	1,266 (30.8)		
≥ 50	105 (51.7)	2,838 (69.2)		
Physical Health Score			0.95	0.94, 0.97
< 50	103 (50.7)	997 (24.3)		
≥ 50	100 (49.3)	3,107 (75.7)		

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime (past three months). OR=odds ratio. CI=confidence interval.

[2] Odds ratio for the association of each variable in the first column on irritable bowel syndrome status. These analyses are adjusted for non-independence of twins within a pair via generalized estimating equations.

† These models are additionally adjusted for gender as a confounder.

Table 3.5. Sensitivity analysis of the magnitude of the association between IBS and MDD when changing the implied timing of onset between MDD and IBS[1].

Primary Exposure	Outcome	OR [1]	95% CI [1]	Precision	% change	Adjustment Variables[2]
IBS[2]	MDD	1.95	1.44, 2.64	1.8	2.6%	None
MDD[2]	IBS	2.00	1.48, 2.71	1.8	2.5%	None

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime (past three months). MDD=major depressive disorder (past six months). OR=odds ratio. CI=confidence interval.

[2] Models are adjusted for the clustering of twin pairs via generalized estimating equations.

3.8 REFERENCES

1. Mayer EA. Emerging disease model for functional gastrointestinal disorders. Am J Med 1999;107(5A):12S-19S.

2. Schmulson MW, Chang L. Diagnostic approach to the patient with irritable bowel syndrome. Am J Med 1999;107(5A):20S-26S.

3. Maxwell PR, Mendall MA, Kumar D. Irritable bowel syndrome. Lancet 1997;350(9092):1691-5.

4. Boyce PM, Koloski NA, Talley NJ. Irritable bowel syndrome according to varying diagnostic criteria: are the new Rome II criteria unnecessarily restrictive for research and practice? Am J Gastroenterol 2000;95(11):3176-83.

5. Kay L, Jorgensen T, Jensen KH. The epidemiology of irritable bowel syndrome in a random population: prevalence, incidence, natural history and risk factors. J Intern Med 1994;236(1):23-30.

6. Talley NJ, Gabriel SE, Harmsen WS, Zinsmeister AR, Evans RW. Medical costs in community subjects with irritable bowel syndrome. Gastroenterology 1995;109(6):1736-41.

7. Hu WH, Wong WM, Lam CL, Lam KF, Hui WM, Lai KC, et al. Anxiety but not depression determines health care-seeking behaviour in Chinese patients with dyspepsia and irritable bowel syndrome: a population-based study. Aliment Pharmacol Ther 2002;16(12):2081-8.

8. Mearin F, Badia X, Balboa A, Baro E, Caldwell E, Cucala M, et al. Irritable bowel syndrome prevalence varies enormously depending on the employed diagnostic criteria: comparison of Rome II versus previous criteria in a general population. Scand J Gastroenterol 2001;36(11):1155-61.

9. Saito YA, Locke GR, Talley NJ, Zinsmeister AR, Fett SL, Melton LJ, 3rd. A comparison of the Rome and Manning criteria for case identification in epidemiological investigations of irritable bowel syndrome. Am J Gastroenterol 2000;95(10):2816-24.

10. Talley NJ, Boyce PM, Jones M. Predictors of health care seeking for irritable bowel syndrome: a population based study. Gut 1997;41(3):394-8.

11. Talley NJ, Howell S, Poulton R. The irritable bowel syndrome and psychiatric disorders in the community: is there a link? Am J Gastroenterol 2001;96(4):1072-9.

12. Kwan AC, Hu WH, Chan YK, Yeung YW, Lai TS, Yuen H. Prevalence of irritable bowel syndrome in Hong Kong. J Gastroenterol Hepatol 2002;17(11):1180-6.

13. Masud MA, Hasan M, Khan AK. Irritable bowel syndrome in a rural community in Bangladesh: prevalence, symptoms pattern, and health care seeking behavior. Am J Gastroenterol 2001;96(5):1547-52.

14. Rome II: the functional gastrointestinal disorders: diagnosis, pathophysiology, and treatment: a multinational consensus. 2nd ed. McLean, VA: Degnon Associates; 2000.

15. Brandt LJ, Bjorkman D, Fennerty MB, Locke GR, Olden K, Peterson W, et al. Systematic review on the management of irritable bowel syndrome in North America. Am J Gastroenterol 2002;97(11 Suppl):S7-26.

16. Thompson WG. The treatment of irritable bowel syndrome. Aliment Pharmacol Ther 2002;16(8):1395-406.

17. Lau EM, Chan FK, Ziea ET, Chan CS, Wu JC, Sung JJ. Epidemiology of irritable bowel syndrome in Chinese. Dig Dis Sci 2002;47(11):2621-4.

18. Blanchard EB, Scharff L, Schwarz SP, Suls JM, Barlow DH. The role of anxiety and depression in the irritable bowel syndrome. Behav Res Ther 1990;28(5):401-5.

19. Swiatkowski M, Rybakowski JK. Depression and T lymphocytes in patients with irritable bowel syndrome. J Affect Disord 1993;28(3):199-202.

20. Mayer EA, Craske M, Naliboff BD. Depression, anxiety, and the gastrointestinal system. J Clin Psychiatry 2001;62 Suppl 8:28-36; discussion 37.

21. Lydiard RB. Irritable bowel syndrome, anxiety, and depression: what are the links? J Clin Psychiatry 2001;62 Suppl 8:38-45; discussion 46-7.

22. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? Gastroenterology 2002;122(4):1140-56.

23. Walker EA, Katon WJ, Jemelka RP, Roy-Bryne PP. Comorbidity of gastrointestinal complaints, depression, and anxiety in the Epidemiologic Catchment Area (ECA) Study. Am J Med 1992;92(1A):26S-30S.

24. Masand PS, Kaplan DS, Gupta S, Bhandary AN, Nasra GS, Kline MD, et al. Major depression and irritable bowel syndrome: is there a relationship? J Clin Psychiatry 1995;56(8):363-7.

25. Garakani A, Win T, Virk S, Gupta S, Kaplan D, Masand PS. Comorbidity of irritable bowel syndrome in psychiatric patients: a review. Am J Ther 2003;10(1):61-7.

26. Vandvik PO, Wilhelmsen I, Ihlebaek C, Farup PG. Comorbidity of irritable bowel syndrome in general practice: a striking feature with clinical implications. Aliment Pharmacol Ther 2004;20(10):1195-203.

27. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. Aliment Pharmacol Ther 2003;17(5):643-50.

28. Anderson LS, Beverly WT, Corey LA, Murrelle L. The Mid-Atlantic Twin Registry. Twin Res 2002;5(5):449-55.

29. Botkin J. Protecting the privacy of family members in survey and pedigree research. Jama 2001;285(2):207-11.

30. Mathews J. Father's Complaints Shut Down Research; U.S. Agencies Act On Privacy Concerns. The Washington Post 2000;Sect. B.07.

31. Furberg H, Olarte M, Afari N, Goldberg J, Buchwald D, Sullivan PF. The prevalence of self-reported chronic fatigue in a U.S. twin registry. J Psychosom Res 2005;59(5):283-90.

32. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. The lifetime history of major depression in women. Reliability of diagnosis and heritability. Arch Gen Psychiatry 1993;50(11):863-70.

33. Kendler KS, Pedersen NL, Neale MC, Mathe AA. A pilot Swedish twin study of affective illness including hospital- and population-ascertained subsamples: results of model fitting. Behav Genet 1995;25(3):217-32.

34. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, D.C.: American Psychiatric Association; 1994.

35. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33(2):160-72.

36. Kato K, Sullivan PF, Evengard B, Pedersen NL. Importance of genetic influences on chronic widespread pain. submitted.

37. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121(12):953-9.

38. Evengard B, Jacks A, Pedersen NL, Sullivan PF. The epidemiology of chronic fatigue in the Swedish Twin Registry. Psychol Med 2005;35(9):1317-26.

39. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34(3):220-33.

40. Stokes ME, Davis CS, Koch GG. Categorical Data Analysis Using the SAS System. 2nd Edition ed. Cary, NC: SAS Institute Inc.; 2000.

41. SAS/STAT Software: Version 8.2. In. Cary, NC: SAS Institute Inc.; 2001.

42. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999;10(1):37-48.

43. Kleinbaum D. Logistic Regression: A self-learning text. New York, NY: Springer-Verlag; 1994.

44. Chey WD, Olden K, Carter E, Boyle J, Drossman D, Chang L. Utility of the Rome I and Rome II criteria for irritable bowel syndrome in U.S. women. Am J Gastroenterol 2002;97(11):2803-11.

45. Viera AJ, Hoag S, Shaughnessy J. Management of irritable bowel syndrome. Am Fam Physician 2002;66(10):1867-74.

46. Thompson WG, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. Dig Dis Sci 2002;47(1):225-35.

47. Sperber AD, Shvartzman P, Friger M, Fich A. Unexpectedly low prevalence rates of IBS among adult Israeli Jews. Neurogastroenterol Motil 2005;17(2):207-11.

48. Hui WM, Shiu LP, Lam SK. The perception of life events and daily stress in nonulcer dyspepsia. Am J Gastroenterol 1991;86(3):292-6.

49. Hahn BA, Kirchdoerfer LJ, Fullerton S, Mayer E. Patient-perceived severity of irritable bowel syndrome in relation to symptoms, health resource utilization and quality of life. Aliment Pharmacol Ther 1997;11(3):553-9.

50. Hahn BA, Yan S, Strassels S. Impact of irritable bowel syndrome on quality of life and resource use in the United States and United Kingdom. Digestion 1999;60(1):77-81.

51. Luscombe FA. Health-related quality of life and associated psychosocial factors in irritable bowel syndrome: a review. Qual Life Res 2000;9(2):161-76.

52. Frank L, Kleinman L, Rentz A, Ciesla G, Kim JJ, Zacker C. Health-related quality of life associated with irritable bowel syndrome: comparison with other chronic diseases. Clin Ther 2002;24(4):675-89; discussion 674.

53. Li FX, Patten SB, Hilsden RJ, Sutherland LR. Irritable bowel syndrome and health-related quality of life: a population-based study in Calgary, Alberta. Can J Gastroenterol 2003;17(4):259-63.

54. Halder SL, Locke GR, 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ, 3rd. Impact of functional gastrointestinal disorders on health-related quality of life: a population-based case-control study. Aliment Pharmacol Ther 2004;19(2):233-42.

55. Aaron LA, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. Arch Intern Med 2000;160(2):221-7.

56. Azpiroz F, Dapoigny M, Pace F, Muller-Lissner S, Coremans G, Whorwell P, et al. Nongastrointestinal disorders in the irritable bowel syndrome. Digestion 2000;62(1):66-72.

57. Aaron LA, Herrell R, Ashton S, Belcourt M, Schmaling K, Goldberg J, et al. Comorbid clinical conditions in chronic fatigue: a co-twin control study. J Gen Intern Med 2001;16(1):24-31.

58. Gomborone JE, Gorard DA, Dewsnap PA, Libby GW, Farthing MJ. Prevalence of irritable bowel syndrome in chronic fatigue. J R Coll Physicians Lond 1996;30(6):512-3.

59. Veale D, Kavanagh G, Fielding JF, Fitzgerald O. Primary fibromyalgia and the irritable bowel syndrome: different expressions of a common pathogenetic process. Br J Rheumatol 1991;30(3):220-2.

60. Pace F, Sarzi-Puttini P, Manzionna G, Molteni P, Turiel M, Panni B, et al. Visceral hypersensitivity is not a feature of fibromyalgia syndrome. Journal of Musculoskeletal Pain 2001;9(1):47-55.

61. Sperber AD, Atzmon Y, Neumann L, Weisberg I, Shalit Y, Abu-Shakrah M, et al. Fibromyalgia in the irritable bowel syndrome: studies of prevalence and clinical implications. Am J Gastroenterol 1999;94(12):3541-6.

62. Aaron LA, Buchwald D. A review of the evidence for overlap among unexplained clinical conditions. Ann Intern Med 2001;134(9 Pt 2):868-81.

63. Hudson JI, Goldenberg DL, Pope HG, Jr., Keck PE, Jr., Schlesinger L. Comorbidity of fibromyalgia with medical and psychiatric disorders. Am J Med 1992;92(4):363-7.

64. Saito YA, Schoenfeld P, Locke GR, 3rd. The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol 2002;97(8):1910-5.

65. Locke GR, 3rd, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ. Risk factors for irritable bowel syndrome: role of analgesics and food sensitivities. Am J Gastroenterol 2000;95(1):157-65.

66. Simren M, Mansson A, Langkilde AM, Svedlund J, Abrahamsson H, Bengtsson U, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion 2001;63(2):108-15.

67. Lu CL, Chen CY, Chang FY, Lee SD. Characteristics of small bowel motility in patients with irritable bowel syndrome and normal humans: an Oriental study. Clin Sci (Lond) 1998;95(2):165-9.

68. Talley NJ, Howell S, Poulton R. Obesity and chronic gastrointestinal tract symptoms in young adults: a birth cohort study. Am J Gastroenterol 2004;99(9):1807-14.

69. Baretic M, Bilic A, Jurcic D, Mihanovic M, Sunic-Omejc M, Dorosulic Z, et al. Epidemiology of irritable bowel syndrome in Croatia. Coll Antropol 2002;26 Suppl:85-91.

70. Camilleri M, Heading RC, Thompson WG. Clinical perspectives, mechanisms, diagnosis and management of irritable bowel syndrome. Aliment Pharmacol Ther 2002;16(8):1407-30.

71. Hungin AP, Chang L, Locke GR, Dennis EH, Barghout V. Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. Aliment Pharmacol Ther 2005;21(11):1365-75.

72. Yilmaz S, Dursun M, Ertem M, Canoruc F, Turhanoglu A. The epidemiological aspects of irritable bowel syndrome in Southeastern Anatolia: a stratified randomised community-based study. Int J Clin Pract 2005;59(3):361-9.

73. Dapoigny M, Bellanger J, Bonaz B, Bruley des Varannes S, Bueno L, Coffin B, et al. Irritable bowel syndrome in France: a common, debilitating and costly disorder. Eur J Gastroenterol Hepatol 2004;16(10):995-1001.

74. Gwee KA, Wee S, Wong ML, Png DJ. The prevalence, symptom characteristics, and impact of irritable bowel syndrome in an asian urban community. Am J Gastroenterol 2004;99(5):924-31.

75. Xiong LS, Chen MH, Chen HX, Xu AG, Wang WA, Hu PJ. A population-based epidemiologic study of irritable bowel syndrome in South China: stratified randomized study by cluster sampling. Aliment Pharmacol Ther 2004;19(11):1217-24.

76. Kanazawa M, Endo Y, Whitehead WE, Kano M, Hongo M, Fukudo S. Patients and nonconsulters with irritable bowel syndrome reporting a parental history of bowel problems have more impaired psychological distress. Dig Dis Sci 2004;49(6):1046-53.

77. Celebi S, Acik Y, Deveci SE, Bahcecioglu IH, Ayar A, Demir A, et al. Epidemiological features of irritable bowel syndrome in a Turkish urban society. J Gastroenterol Hepatol 2004;19(7):738-43.

78. Hoseini-Asl MK, Amra B. Prevalence of irritable bowel syndrome in Shahrekord, Iran. Indian J Gastroenterol 2003;22(6):215-6.

79. Hillila MT, Farkkila MA. Prevalence of irritable bowel syndrome according to different diagnostic criteria in a non-selected adult population. Aliment Pharmacol Ther 2004;20(3):339-45.

80. Saito YA, Talley NJ, L JM, Fett S, Zinsmeister AR, Locke GR. The effect of new diagnostic criteria for irritable bowel syndrome on community prevalence estimates. Neurogastroenterol Motil 2003;15(6):687-94.

4 RESULTS MANUSCRIPT 2. IRRITABLE BOWEL SYNDROME: A COTWIN-CONTROL ANALYSIS

4.1 ABSTRACT

Background. Irritable bowel syndrome (IBS) and major depressive disorder (MDD) are common disorders with one-year prevalences ranging from 10-20% in the population. A large proportion of individuals who satisfy diagnostic criteria for one of these disorders also tend to satisfy criteria for the other. While MDD has a well-characterized familial tendency; this tendency is less substantiated among individuals meeting criteria for IBS. Further, among individuals who satisfy diagnostic criteria for both disorders, even less is known about the role of familial tendency and environmental influences to disease susceptibility.

Aim. To examine the genetic and environmental architecture of the co-occurrence of MDD and IBS in participants from the population-based Swedish Twin Registry.

Methods. We implemented a nested case-control study and a co-twin control study. IBS cases were ascertained using an adapted version of the Rome criteria and MDD cases were assessed using a shortened version of the

Computerized International Diagnostic Interview. The case-control study included individuals with complete covariate information (N=29,616), and adjusted for twin pair, 3-year age band, and sex. The co-twin control study employed 288 twin pairs discordant for IBS. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using generalized estimating equations for the case-control study and conditional logistic regression for the co-twin control study.

Findings. In both studies, co-morbid disorders of chronic widespread pain, chronic fatigue-like illness, and MDD were more common in IBS cases than controls. In the case-control study, a positive association between MDD and IBS was noted, where individuals with MDD had an increased odds of IBS (OR =2.7; 95% CI 2.3, 3.2). In the co-twin study, the association was similar (OR=2.2; 95% CI 1.5, 3.3).

Interpretation. Based on this analysis, genetic and environmental factors do not confound the association between MDD and IBS. Rather it appears as though one of these disorders is part of the disease causing sequence of events for the other disorder. However, the study design makes it impossible to evaluate the timing of onset between MDD and IBS.

4.2 INTRODUCTION

Irritable bowel syndrome (IBS) affects 10-20% of the adult population (1, 2). IBS is a complex trait with a demonstrated familial tendency (genetic or environmental); documented through family (3, 4) and twin studies (5-7). Similarly, major depressive disorder (MDD) affects 10-20% of the adult population (8-11). MDD is also a complex trait influenced by both genes and environment. Although no genes evaluated have been substantiated, further examination of the candidate genes (12-15) involved in MDD has been undertaken. Additionally, family (16-20), adoption (16, 21, 22), twin (16, 23, 24), and linkage (25, 26) studies support a familial predisposition to MDD. Research has also demonstrated that 50% of individuals with IBS also have a psychiatric disorder, with MDD being the most prevalent (27, 28).

The co-occurrence of MDD and IBS has been understudied. Moreover, the research that has been conducted is limited by the use of patient populations (29, 30). The use of a patient population could induce a bias as those that actively seek care may be different that those that do not seek care for their disorders. In particular, individuals seeking treatment for IBS related symptoms document a more depressed psychopathology than individuals not seeking care (28, 30). Thus, the results may be biased and reflective of those individuals that are actively seeking treatment for either or

both disorders. In the few studies that have used population-based samples, the co-occurrence between the two disorders is smaller. One study documented 13% of individuals with two gastrointestinal symptoms also had MDD (31). Similarly, a second population-based study found that 13% of individuals with Rome II IBS had chronic depression, however the odds of IBS was not increased among those with MDD, thereby not substantiating any association between IBS and MDD (32). However it is difficult to compare these study results because different symptom criteria for IBS and MDD were employed, different geographic populations were used, and different measurement tools were employed.

Regardless of the discrepancy in the magnitude of the overlap in occurrence between IBS and MDD between patient and population samples, it is clear that their co-occurrence happens more often than would be expected. Thus the question becomes why is this occurring, or more directly, what is causing this phenomenon. This is an especially daunting task since the biologic mechanisms of occurrence of both IBS and MDD are not fully known. However, since both disorders separately demonstrate familiality, an etiologic hypothesis of a possible common familiality to their co-occurrence is plausible. Further, if these disorders do share a common biology, it may be possible to manage these disorders with medications specifically targeted to the common biology, or to obtain an additional indication for a medication already being used to treat one of these disorders. This would not only aid in

the disease management of individuals, but also in improving our understanding of their disease etiology.

In this study, we employ a co-twin control design. This analysis exploits the natural biologic similarity and differing degrees of genetic relatedness among monozygotic (MZ) and dizygotic (DZ) twins to assess the importance of potential risk factors after controlling for genetic and familial environmental effects (33, 34). Specifically, through the use of this study design in combination with a case-control study, it is possible to assess if familial environment or genetics are important to the etiology of co-occurring disorders or if there is a biologic causal mechanism between the co-occurring disorders. This is performed by comparing results from the two study designs. If an association is observed between MDD and IBS in the case control study and also when the non-diseased co-twin is used as a control for each case (diseased co-twin), the association is not fully explained by genetic or familial environmental factors and therefore the results support a causal link between MDD and IBS (33-35). However if an association is found in the case control study but not in the co-twin control study, then the case control study findings reflect confounding by genetic and familial environmental factors (34, 35).

Using this study design, we aim to examine the genetic and environmental architecture of the co-occurrence of MDD and IBS in

participants from the population-based Swedish Twin Registry. To accomplish this goal we assessed the association between MDD and IBS using a case-control design. We then compared these results to those of a co-twin control study.

4.3 METHODS

4.3.1 Study Population

We conducted a nested case-control study on a subset of the Swedish Twin Registry (STR, http://www.mep.ki.se/twinreg/index_en.html). The STR cohort is the largest population-based registry of twin births (33, 36). Over a four-year period ending in December 2002, all living, contactable, interviewable, and consenting twins in the STR born between 1/1/1935 and 12/31/1958 were screened for a range of disorders that included gastrointestinal and mental disorders as well as self-rated health, and comprised the Screening Across the Lifespan Twin (SALT) study of the STR. A second study is currently targeting younger adult twins.

4.3.2 Data Collection

During this time, approximately 1,000 pairs were randomly selected each month for interviews. Prior to interviewing, a letter describing the study was sent to these twins, and then telephone contact and interviews with the twins commenced within two weeks of the initial letter. Interviews were conducted by trained interviewers using a computer based data collection

system. This process was repeated until all twins were interviewed or had actively or passively declined to participate. The study was reviewed and approved by the Ethical Committee of the Karolinska Institutet, the Swedish Data Inspection Board, and the Institutional Review Board of the University of North Carolina at Chapel Hill.

4.3.3 Irritable Bowel Syndrome (IBS) Cases

Gastrointestinal disorders were assessed during the interview using an adapted version of the Rome criteria. This Rome II IBS definition included a positive response to the following questions: recurrent abdominal discomfort, abdominal discomfort that lasted as least 7 days a month, if intestinal problems were more prominent when feces became looser and defecation more frequent, and reporting recurrent problems with pain in either the upper abdomen, lower abdomen, or another part of the abdomen. Participants of the SALT study who reported a history of Crohn's disease (n=34), ulcerative colitis (n=55), stomach ulcers (n=59), intestinal ulcers (n=46), or any combination of these (n=52) were excluded. Together, the subset of questions combined with the exclusions was applied to the cohort to identify Rome II IBS cases in the SALT study.

To assess the magnitude of agreement between our derived IBS definition and that of other studies, we compared our IBS definition with a Rome II IBS definition used in the Adult Health and Personality (AHP) Study

of the Mid-Atlantic Twin Registry (37). This study was chosen because we had access to the questionnaire and raw data for the AHP study. Briefly, the questions used in the SALT were matched to questions in the AHP questionnaire. Then, using the AHP data, we determined those satisfying IBS symptom criteria using the Rome II definition of the AHP study and also those satisfying the subset of questions corresponding to the SALT IBS definition we described above. Comparison of these definitions was done to determine concordance between the IBS definitions and reliability of the IBS definition. Results of the comparison in IBS definition yielded a good to excellent reliability (κ=0.92), and 99% concordance in IBS case status.

4.3.4 Exposure Assessment

During the interview, emphasis was placed on diagnostic items for determining whether a twin was likely to have a disease, rather than asking a twin directly about the disease. The diagnostic items were presented in a branching format so that within a disease area, follow-up items were asked only if the participant responded positively to the key introductory items. These diagnostic items were compiled by experts in each of the disease areas. Additionally, standardized instruments were used when available, for example a specific short version of the Computerized International Diagnostic Interview (CIDI) (38) was used for psychiatric disorders.

Irritable bowel syndrome (IBS) was assessed by an adapted version of the Rome criteria (7, 39). Major depressive disorder (MDD) (40) and generalized anxiety disorder (GAD) (40) were assessed using the short Computerized International Diagnostic Interview (CIDI) through which Diagnostic and Statistical Manual of Mental Disorders, third edition revised (DSM-III-R) (41) diagnoses could be obtained. Although no psychological evaluations were performed, the interview used a validated and appropriate instrument to assess MDD using a personal interview (38) which has been implemented in several population based studies of MDD (42-44). An adaptation of the American College of Rheumatology criteria for fibromyalgia (45, 46) was used to assess chronic widespread pain (CWP). Chronic fatigue-like illness was assessed using a module similar to the US Centers for Disease Control (CDC) consensus criteria (47) for chronic fatigue syndrome (48). Questionnaire items developed for the Older Americans Resource Survey at Duke University (49) were used to assess self-rated health.

To establish zygosity of the twin pairs, questions about childhood resemblance were used. Validation studies have demonstrated this technique to accurately determine zygosity 98% of the time (33). Additional confirmation of the zygosity determination was performed on a subset of this sample using 13 DNA markers. Zygosity diagnosis using the questions agreed with the DNA marker zygosity determination in 99% of the pairs (n=199 twin pairs) (33).

4.3.5 Statistical Analyses

Odds ratios (OR) with 95% confidence intervals (CI) were used to estimate the association between MDD and IBS, and were obtained using regression models in the SAS software (50). For the case-control study, ORs were calculated using generalized estimating equations in SAS (PROC GENMOD), adjusted for twin pair, sex, and age (3 year age band). ORs were calculated for the co-twin control subjects using conditional logistic regression in SAS (PROC PHREG) where the unit of analysis was the twin pair.

4.3.6 Case-control Analysis

A nested case-control study was employed to assess the association between MDD and IBS in this population of twins. Generalized estimating equations (GEE) were used to obtain odds ratios (OR) and 95% confidence intervals (CI) for the MDD-IBS association, thereby adjusting for the familial clustering of twin data. Additionally we adjusted the analysis for gender and age (3-year age band).

Multivariable Model. In the case-control study, a multivariable model using both sexes was constructed using a backward elimination procedure (described below). This model was subsequently used for the co-twin control study to be able to compare the study results. To determine the appropriate model we first assessed effect measure modification and then confounding. At the end of the confounding assessment, the final fully adjusted

multivariable model assessing the association between MDD and IBS in this population was obtained.

Effect Measure Modification. To assess effect measure modification of the association between MDD and IBS, a logistic regression model (using GEE to control for twin relatedness) was constructed using all first-order product interactions between MDD and all other covariates deemed as potential confounders of the MDD-IBS association based on an evaluation of a directed acyclic graph (51). To determine if any potential interactions were significant effect modifiers, we compared results from two logistic regression models, one with all the potential interactions and one without any interaction terms, both containing the main exposure and all linear terms of the interactions. This allowed for a 'chunk' test using the likelihood ratio test and its associated χ^2 statistic to determine if the interaction terms as a whole could be eliminated from the model (52). If the χ^2 statistic associated with this test was not significant at $p<0.20$, the removed interaction terms were not significant to the MDD-IBS association and thus remained eliminated from the model (52). However, if the χ^2 statistic associated with this 'chunk' test for interaction was significant at $p<0.20$, at least some or all of the interaction terms were significant. Thus all the interaction terms were returned to the model for further assessment using backward elimination to examine each interaction term one at a time in order to eliminate insignificant variables from the model (52). Once all potential effect modifiers were assessed, covariates

involved in significant effect modification terms were included as linear terms in all remaining logistic models.

Confounding. Once the assessment of effect measure modification was complete, we assessed confounding for covariates not involved in effect measure modification terms. To determine if a potential confounder of the MDD-IBS association did confound the association in our population, logistic regression models (using generalized estimating equations) with and without the potential confounder were compared using the OR for MDD. First, we obtained results for the model with the exposure, all potential confounders, and any significant effect measure modification terms (full model). Using these results, the potential confounder not involved in any effect measure modification terms with the largest associated p-value was removed from the full model and we obtained modeling results for this adjusted model. In order to determine if the inclusion of this term confounded the association, the OR from this adjusted model was compared to the OR from the full model. If $\ln|(OR_{full}/OR_{adjusted})| > 0.10$, then the variable was a confounder and was returned to the full model; otherwise the variable was not a confounder of the MDD-IBS association and was eliminated from the model (52). This process was repeated for all potential confounders, where the full model changed based on the results of the prior confounder assessment (i.e. if the potential confounder did not confound the association, then that model became the full model), until all potential confounders were assessed.

4.3.7 Co-Twin Control Study, MZ and DZ Twin Pairs

Upon completion of the case-control analysis, we performed a co-twin control analysis using both MZ and DZ twin pairs that were discordant for IBS (N=288 twin pairs). Thus the healthy (no IBS) co-twins were used as the controls for the diseased (IBS) twins. This allowed for an evaluation of potential confounding by the latent variable of unmeasured early familial environment. To easily compare the results of the case-control and co-twin control analyses, the covariates from the final fully adjusted multivariable model from the case-control study were used.

4.3.8 Co-Twin Control Study, MZ Twin Pairs

This extension of the co-twin analysis restricted the sample to only MZ twin pairs that were discordant for IBS (N=119 twin pairs). Rationale for this analysis was to control for confounding due to genetic effects when compared to the co-twin control results using both MZ and DZ twin pairs. Adjustment variables were the same as those used in the case-control study.

4.4 RESULTS

4.4.1 Sample Description

Of all eligible twins (N=41,499), 31,406 individual twins responded, giving an individual response rate of 75.7%. This included data from 12,407 complete pairs and 6,592 incomplete pairs. Zygosity of the complete pairs was determined, and the sample consisted of 3,269 monozygotic pairs, 9,010

dizygotic pairs, and 128 pairs of unknown zygosity. This analysis focused on the 29,616 individual twins who had complete covariate information. Based on the χ^2 statistics (results not shown), missing data for the variables we examined did not differ by IBS status. These twins represented 119 monozygotic twin pairs and 169 same-sex dizygotic twin pairs discordant for IBS.

4.4.2 Descriptive Analyses

Table 4.1 shows descriptive statistics of cases and controls irrespective of sex. There were no differences in body mass index or age between cases and controls. More women satisfied criteria for IBS than men. Co-morbid disorders of CWP, chronic fatigue-like illness, and MDD, were more common in cases than in controls, with positive associations and precise estimates (confidence limit ratio (CLR) <2.0 (53)). Control responses to the health status questions demonstrated better self-rated health compared to cases. Table 4.2 describes the descriptive statistics of IBS cases and controls for the co-twin control study. In the twin pair analyses, covariates were distributed similarly to their distributions in the case control study.

4.4.3 Case-Control Model Selection

Effect Measure Modification. When we assessed the first-order product interactions between MDD and the potential confounding covariates

as potential effect measure modifiers, no interactions significantly modified the association between MDD and IBS.

Confounding. Since we did not identify any effect measure modifiers, the covariates identified as potential confounders of the MDD-IBS association were assessed. In this analysis none of the remaining covariates investigated confounded the association between MDD and IBS.

4.4.4 Case-Control Results

Multivariable Model. The multivariable model investigating the association between MDD and IBS was adjusted for sex and age at interview (3-year age band) (Table 4.3). The association between MDD and IBS was similar to the crude association, but slightly attenuated.

4.4.5 Co-Twin Control Results

MZ and DZ Twin Pairs. When the healthy co-twin was used as the control for the IBS affected twin, the association between MDD and IBS was similar to the case control estimate (Table 4.3).

MZ Twin Pairs. When only IBS discordant MZ twin pairs were used, the association between MDD and IBS was larger in magnitude than either the case-control study or the co-twin study among MZ and DZ twins. While it was less precise, the confidence interval encompassed all previous estimates of the MDD-IBS associations demonstrated in our analyses (Table 4.3).

4.5 DISCUSSION

We evaluated familial predisposition to co-occurring MDD and IBS in 29,616 members of the population-based Swedish Twin Registry aged 42-64 years, comprising part of the SALT study. To accomplish this goal, we implemented a three-step analysis design. The first study was a classic case-control study examining the association between MDD and IBS in this general population sample. This study confirmed that individuals who were female, or had co-morbid disorders of MDD, CWP, and chronic fatigue-like illness, were more likely to satisfy diagnostic criteria for IBS. In the multivariable analysis of IBS, individuals with MDD had a higher likelihood of having IBS (OR 2.7; 95% CI 2.3, 3.2). In the co-twin study, using both MZ and DZ IBS discordant twin pairs, there was also an increased odds of IBS among those with MDD (OR 2.2; 95% CI 1.5, 3.3). In the third study, when only MZ IBS discordant twin pairs were used, an imprecise increased odds of IBS among those with MDD was demonstrated (OR 3.2; 95% CI 1.7, 6.1).

In our study, women had a higher prevalence of IBS compared to men. This difference in prevalence signifies that women are affected at a ratio of about 1.8:1 compared to men in this study. Previous studies have demonstrated women to have IBS at a rate of 2:1 compared to men (1, 54, 55). Thus while our estimate is slightly smaller in magnitude, it is similar to the 2:1 ratio of female to male IBS affection seen in prior studies, further

evidence that our twin population is similar to singleton populations in terms of the IBS gender distribution.

In this subset of the STR, 11% of individuals with IBS also reported symptoms consistent with chronic widespread pain. Prior studies that employed the Rome I symptom criteria for IBS have documented higher rates of co-occurrence between IBS and CWP (56-58). This discrepancy in the magnitude may be due to our use of an approximation of the Rome II criteria for IBS which may be more accurate (59) and has been associated with lower prevalence rates for IBS (60, 61) than earlier symptom criteria (32, 62-64). However, regardless of the lower magnitude demonstrated in our study, it is still larger than the background rate of CWP of 2.4% in this study and thus adds to the literature in support of a larger proportion of individuals fulfilling symptoms of both disorders than either disorder independently.

Similarly, our study documented 12% of individuals with IBS also fulfilled the criteria for chronic fatigue-like illness, and conversely 11% of individuals with chronic fatigue-like illness also fulfilled IBS criteria. Most prior studies examining this relationship have used the Manning criteria for IBS (58, 65). In these studies, the rate of IBS among chronic fatigue syndrome patients was between 50 and 92% (58, 66). Our rates, while higher than population prevalences for either disorder independently, are lower than those previously documented, which could be attributed to our use of a

general population as opposed to patient populations used in earlier studies. The use of patient populations is problematic as it involves individuals that are self-selecting to use healthcare and may not be representative of the general population. As has been demonstrated for IBS, patient populations are not similar to non-patient populations (28, 30). Therefore, in our general population, while the magnitude of the association is lower, it demonstrates that chronic fatigue-like illness and IBS occur more often together than as individual disorders amongst those that do and do not seek health care.

In our study, 45% of individuals satisfying the Rome II IBS criteria also satisfied the symptom criteria for MDD. Prior studies have documented IBS patients to be more depressed than inflammatory bowel disease patients or healthy controls using the Anxiety Disorders Interview Schedule—Revised (ADIS-R) (30). IBS patients also had higher scores on the Zung Depression Self-Rating Scale compared to controls (67). The study by Mayer et al., summarized studies of IBS patients seeking care at gastroenterology clinics and found that in studies with an adequate sample size and a standardized psychiatric interview, 50-60% of IBS patients also have psychiatric disorders (27). Additionally among IBS patients seeking specialist care, 54-94% met DSM-III-R criteria for a primary psychological disorder (29), however in a population based study, only 13% of IBS patients met DSM-III criteria for a primary psychiatric disorder (31). Thus among individuals with IBS that actively seek healthcare, the percentage of individuals satisfying some sort of

major depression criteria is large, but less is known about individuals in the community. This study begins to address the co-morbidity of IBS and MDD in a population-based sample.

In the prior population-based study examining the co-occurrence of IBS and MDD, the overlap between these two disorders was 13%, while ours was 45%. Reasons for this discrepancy could be the use of different symptom criteria for IBS (Rome II versus Manning) and different MDD diagnostic criteria. However one would think that the study using the Manning criteria would have a larger potential overlap of individuals suffering form MDD since the Manning criteria are less restrictive and identify a more heterogeneous group of IBS sufferers. The overlap between IBS and MDD in our study was more similar to those reported using patient populations, and thus more research into the co-occurrence of IBS and MDD in the general population is warranted.

This study supports an association between MDD and IBS. Previous population-based studies of the association between MDD and IBS have been inconsistent (28, 32, 68). The Masand et al. study supported an association between MDD and IBS among those seeking some sort of healthcare. However this study was mainly descriptive and did not use acknowledged symptom criteria for IBS (68). Similarly, the Lydiard et al. study supported an association between MDD and IBS in a population-based

sample, yet gastrointestinal symptoms were assessed in a structured psychiatric interview not designed to diagnose IBS (28). Additionally, the IBS definition was an “IBS-like” cluster of gastrointestinal symptoms, not corresponding to any particular symptom criteria for IBS (28). In the population-based study by Talley et al., both IBS and MDD were evaluated using validated questionnaires and appropriate symptom criteria for IBS (Manning and Rome II). This study did not support an association between MDD and IBS, however it was limited to younger individuals, aged 26 years old, and thus did not include those that have gone through the ages where the prevalence of MDD increases (namely at ages 30 and 50) (32). Additionally, the stratified analysis based on Rome II criteria was underpowered.

The results from the co-twin control study suggest that there is a common causal link between MDD and IBS. Since both MDD and IBS have unknown etiologies, it is possible that one of these disorders may predispose individuals to the other disorder. Additionally the use of serotonin derived drugs to treat both MDD and IBS (69, 70) supports a common biologic similarity between these disorders. Our results support the notion that either IBS or MDD plays an etiologic role in the occurrence of the other disorder, and encourage future research into the etiology of the co-occurrence of IBS and MDD in a general population.

This study has several strengths. First, the SALT population contains both patients seeking care for their IBS symptoms and those who do not. This is important because previous studies have shown that IBS patients who seek care are different from those who do not, specifically in their psychological profiles, with those seeking care having more mental illness (28, 30). Second, this study was conducted in a large population-based sample of twins. Because this study was population based, the potential for ascertainment bias was likely reduced because participants' awareness of their disease status should have remained small since it could not be determined which disease was the main disease of interest based on the wide array of diseases contained in the interview. Third, this study applied two analytical strategies in an attempt to disentangle the complex genetic and environmental influences on the co-occurrence of MDD and IBS.

Despite these strengths there were some limitations. The main limitation of this study was its cross-sectional nature. All disorders were assessed through a telephone interview at the same time. Thus it is not possible in this study to address the timing of onset between any of these associations. As such, it is only able to compare associations and not imply anything about risk. Another limitation was its reliance on self-report. Specifically, self-reported data may lead to disease misclassification in many ways. Disease misclassification is possible as no psychological evaluations, clinical exams, or medical record reviews were performed to confirm the

disorders we obtained using the interview. Therefore, individual participants could have responded hypervigilantly to questions during the interview, or they could have responded lackadaisically, either option leading to misclassification of disease and a possible bias to our results. Additionally, it is possible that we may have underestimated the number of IBS cases, as we identified a prevalence of IBS of 2%, which is notably lower than that found in other population-based studies of IBS employing Rome II symptom criteria (32, 55, 60-64, 71, 72). However we feel that the amount of misclassification of our data is small due to the use of previously validated research instruments.

In conclusion, the demonstrated association between MDD and IBS in the case-control study was substantiated in the co-twin analysis. This reflects that the association is not due to a general susceptibility factor (genetic or familial-environmental influences) which is shared by MDD and IBS but more likely one of these disorders predisposes individuals to the other disorder. Future studies should examine this relationship in more depth, as we were unable to assess the timing of onset between MDD and IBS, thereby only substantiating an association in a general population.

4.6 ACKNOWLEDGEMENTS

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4.7 TABLES

Table 4.1. Descriptive statistics of IBS case-control population, from the Screening Across the Lifespan Twin Study of the Swedish Twin Registry[1].

Variable	Case-Control Population (n=29,616)		
	Cases, n (%) (n=611)	Controls, n (%) (n=29,005)	OR (95% CI)[1]
Female Sex	390 (63.8)	15,039 (51.8)	1.64 (1.39, 1.93)
Major Depressive Disorder	275 (45.0)	6,227 (21.5)	2.98 (2.53, 3.51)
Chronic Widespread Pain	69 (11.3)	691 (2.4)	5.17 (3.97, 6.73)
Chronic Fatigue-like illness	72 (11.8)	597 (2.1)	6.31 (4.87, 8.18)
Body Mass Index[2]	25.2 (0.14)	25.0 (0.02)	1.02 (0.99, 1.04)
Age at interview[2]	53.7 (0.04)	53.7 (0.04)	0.97 (0.95, 0.98)
Health Status			
Better/same health as 5 years earlier	361 (59.1)	22,469 (77.5)	0.42 (0.36, 0.50)
Health does not limit activities	306 (50.1)	21,667 (74.7)	0.43 (0.36, 0.52)
Health limited usual activities, 0-7 days in prior six months	467 (76.4)	25,600 (88.3)	0.34 (0.29, 0.40)

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime; OR=odds ratio; CI=confidence interval.

[2] Least square mean and standard errors (LS mean (SE)) are presented for continuous characteristics. These were obtained from mixed models that accounted for the relatedness of twin pairs.

Table 4.2. Descriptive statistics of IBS Discordant MZ and DZ twin pairs and IBS Discordant MZ twin pairs from the Screening Across the Lifespan Twin Study of the Swedish Twin Registry[1].

Variable	MZ and DZ IBS Discordant Twins (n=576)			MZ IBS Discordant Twins (n=238)		
	Cases, n (%) (n=288)	Controls, n (%) (n=288)	OR (95% CI)[1]	Cases, n (%) (n=119)	Controls, n (%) (n=119)	OR (95% CI)
Female Sex	191 (66.3)	191 (66.3)	1.0	83 (69.8)	83 (69.8)	1.0
Major Depressive Disorder	130 (45.1)	86 (29.9)	2.22 (1.5, 3.29)	63 (52.9)	37 (31.1)	3.17 (1.66, 6.06)
Chronic Widespread Pain	28 (9.72)	15 (5.21)	2.08 (1.05, 4.15)	14 (11.8)	8 (6.7)	2.20 (0.76, 6.33)
Chronic Fatigue-like illness	37 (12.85)	12 (4.17)	3.50 (1.73, 7.07)	18 (15.1)	9 (7.6)	2.29 (0.94, 5.55)
Body Mass Index[2]	25.06 (0.21)	24.82 (0.21)	1.03 (0.97, 1.10)	25.36 (0.33)	25.34 (0.33)	1.0 (0.90, 1.12)
Age at interview[2]	52.59 (0.35)	52.57 (0.35)	2.25 (0.65, 7.77)	52.94 (0.54)	52.93 (0.54)	1.54 (0.28, 8.57)
Health Status						
Better/same health as 5 years earlier	161 (55.9)	211 (73.3)	0.41 (0.27, 0.60)	64 (53.8)	82 (68.9)	0.40 (0.21, 0.78)
Health does not limit activities	221 (76.7)	243 (84.4)	0.61 (0.40, 0.93)	93 (78.2)	97 (81.5)	0.79 (0.40, 1.55)
Health limited usual activities, 0-7 days in prior six months	147 (51.0)	198 (68.8)	0.45 (0.31, 0.65)	58 (48.7)	75 (63.0)	0.47 (0.25, 0.87)

[1] Abbreviations: MZ=monozygotic; DZ=dizygotic; IBS=irritable bowel syndrome in lifetime; OR=odds ratio; CI=confidence interval.

[2] Least square mean and standard errors (LS Mean (SE)) are presented for continuous characteristics. These were obtained from mixed models that accounted for the relatedness of twin pairs.

Table 4.3. Multivariable analysis of Major Depressive Disorder and its Relation to IBS[1].

Study Population	IBS Cases, n (%)	IBS Controls, n (%)	OR for MDD[1]	95% CI[1]	Adjustment Variables
Crude	611 (2.1)	29,005 (97.9)	2.98	2.53, 3.51	None
IBS Case Control Study	611 (2.1)	29,005 (97.9)	2.73	2.31, 3.23	Gender and age
MZ and DZ IBS Discordant Twin Pairs[1]	288 (50)	288 (50)	2.22	1.50, 3.29	None[2]
MZ IBS Discordant Twin Pairs[1]	119 (50)	119 (50)	3.17	1.66, 6.06	None[2]

[1] Abbreviations: IBS=irritable bowel syndrome in lifetime; MDD=major depressive disorder; OR=odds ratio; CI=confidence interval; MZ=monozygotic; DZ=dizygotic.

[2] These analyses were adjusted for gender and age due to the matching of twin pairs.

4.8 REFERENCES

1. Thompson WG, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. Dig Dis Sci 2002;47(1):225-35.

2. Viera AJ, Hoag S, Shaughnessy J. Management of irritable bowel syndrome. Am Fam Physician 2002;66(10):1867-74.

3. Locke GR, 3rd, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ, 3rd. Familial association in adults with functional gastrointestinal disorders. Mayo Clin Proc 2000;75(9):907-12.

4. Kalantar JS, Locke GR, 3rd, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. Gut 2003;52(12):1703-7.

5. 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(4):799-804.

6. 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(8):1311-7.

7. Svedberg P, Johansson S, Wallander MA, Hamelin B, Pedersen NL. Extra-intestinal manifestations associated with irritable bowel syndrome: a twin study. Aliment Pharmacol Ther 2002;16(5):975-83.

8. Bland RC. Epidemiology of affective disorders: a review. Can J Psychiatry 1997;42(4):367-77.

9. Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, et al. Cross-national epidemiology of major depression and bipolar disorder. Jama 1996;276(4):293-9.

10. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). Jama 2003;289(23):3095-105.

11. Kessler RC, Walters EE. Epidemiology of DSM-III-R major depression and minor depression among adolescents and young adults in the National Comorbidity Survey. Depress Anxiety 1998;7(1):3-14.

12. Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch Gen Psychiatry 2000;57(8):729-38.

13. Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P, et al. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol Psychiatry 1997;2(5):398-402.

14. Hauser J, Leszczynska A, Samochowiec J, Czerski PM, Ostapowicz A, Chlopocka M, et al. Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. Eur Psychiatry 2003;18(3):129-32.

15. Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry 2003;8(6):574-91.

16. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157(10):1552-62.

17. Hammen C, Brennan PA. Severity, chronicity, and timing of maternal depression and risk for adolescent offspring diagnoses in a community sample. Arch Gen Psychiatry 2003;60(3):253-8.

18. Lieb R, Isensee B, Hofler M, Pfister H, Wittchen HU. Parental major depression and the risk of depression and other mental disorders in offspring: a prospective-longitudinal community study. Arch Gen Psychiatry 2002;59(4):365-74.

19. Klein DN, Lewinsohn PM, Seeley JR, Rohde P. A family study of major depressive disorder in a community sample of adolescents. Arch Gen Psychiatry 2001;58(1):13-20.

20. Klein DN, Shankman SA, Lewinsohn PM, Rohde P, Seeley JR. Family study of chronic depression in a community sample of young adults. Am J Psychiatry 2004;161(4):646-53.

21. Wender PH, Kety SS, Rosenthal D, Schulsinger F, Ortmann J, Lunde I. Psychiatric disorders in the biological and adoptive families of adopted individuals with affective disorders. Arch Gen Psychiatry 1986;43(10):923-9.

22. Cadoret RJ, O'Gorman TW, Heywood E, Troughton E. Genetic and environmental factors in major depression. J Affect Disord 1985;9(2):155-64.

23. Fu Q, Heath AC, Bucholz KK, Nelson E, Goldberg J, Lyons MJ, et al. Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. Arch Gen Psychiatry 2002;59(12):1125-32.

24. Kendler KS, Aggen SH. Time, memory and the heritability of major depression. Psychol Med 2001;31(5):923-8.

25. Zubenko GS, Maher B, Hughes HB, 3rd, Zubenko WN, Stiffler JS, Kaplan BB, et al. Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. Am J Med Genet 2003;123B(1):1-18.

26. Holmans P, Zubenko GS, Crowe RR, DePaulo Jr. JR, Scheftner WA, Weissman MM, et al. Genomewide significant linkage to recurrent, early-onset major depressive disorder on chromosome 15q. Am J Hum Genet 2004;74:1154-1167.

27. Mayer EA, Craske M, Naliboff BD. Depression, anxiety, and the gastrointestinal system. J Clin Psychiatry 2001;62 Suppl 8:28-36; discussion 37.

28. Lydiard RB. Irritable bowel syndrome, anxiety, and depression: what are the links? J Clin Psychiatry 2001;62 Suppl 8:38-45; discussion 46-7.

29. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? Gastroenterology 2002;122(4):1140-56.

30. Blanchard EB, Scharff L, Schwarz SP, Suls JM, Barlow DH. The role of anxiety and depression in the irritable bowel syndrome. Behav Res Ther 1990;28(5):401-5.

31. Walker EA, Katon WJ, Jemelka RP, Roy-Bryne PP. Comorbidity of gastrointestinal complaints, depression, and anxiety in the Epidemiologic Catchment Area (ECA) Study. Am J Med 1992;92(1A):26S-30S.

32. Talley NJ, Howell S, Poulton R. The irritable bowel syndrome and psychiatric disorders in the community: is there a link? Am J Gastroenterol 2001;96(4):1072-9.

33. Lichtenstein P, De Faire U, Floderus B, Svartengren M, Svedberg P, Pedersen NL. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. J Intern Med 2002;252(3):184-205.

34. Kujala UM, Kaprio J, Koskenvuo M. Modifiable risk factors as predictors of all-cause mortality: the roles of genetics and childhood environment. Am J Epidemiol 2002;156(11):985-93.

35. Wirdefeldt K, Gatz M, Pawitan Y, Pedersen NL. Risk and protective factors for Parkinson's disease: a study in Swedish twins. Ann Neurol 2005;57(1):27-33.

36. Pedersen NL, Lichtenstein P, Svedberg P. The Swedish Twin Registry in the third millennium. Twin Res 2002;5(5):427-32.

37. Furberg H, Olarte M, Afari N, Goldberg J, Buchwald D, Sullivan PF. The prevalence of self-reported chronic fatigue in a U.S. twin registry. J Psychosom Res 2005;59(5):283-90.

38. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51(1):8-19.

39. Thompson WG, Doterall G, Drossman DA, Heaton KW, Kruis W. Irritable bowel syndrome: guidelines for the diagnosis. Gastroenterology International 1989;2:92-95.

40. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, D.C.: American Psychiatric Association; 1994.

41. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 3rd ed. Washington, DC: American Psychological Association; 1987.

42. Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, et al. Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr Scand Suppl 2004(420):21-7.

43. Spijker J, de Graaf R, Bijl RV, Beekman AT, Ormel J, Nolen WA. Determinants of persistence of major depressive episodes in the general population. Results from the Netherlands Mental Health Survey and Incidence Study (NEMESIS). J Affect Disord 2004;81(3):231-40.

44. Haarasilta LM, Marttunen MJ, Kaprio JA, Aro HM. Correlates of depression in a representative nationwide sample of adolescents (15-19 years) and young adults (20-24 years). Eur J Public Health 2004;14(3):280-5.

45. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33(2):160-72.

46. Kato K, Sullivan PF, Evengard B, Pedersen NL. Importance of genetic influences on chronic widespread pain. submitted.

47. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121(12):953-9.

48. Evengard B, Jacks A, Pedersen NL, Sullivan PF. The epidemiology of chronic fatigue in the Swedish Twin Registry. Psychol Med 2005;35(9):1317-26.

49. The Older Americans Resource Survey. Multidimensional Functional Assessment: The OARS Methodology. Durham, NC: Duke University; 1978.

50. SAS/STAT Software: Version 8.2. In. Cary, NC: SAS Institute Inc.; 2001.

51. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999;10(1):37-48.

52. Kleinbaum D. Logistic Regression: A self-learning text. New York, NY: Springer-Verlag; 1994.

53. Poole C. Low P-values or narrow confidence intervals: which are more durable? Epidemiology 2001;12(3):291-4.

54. Brandt LJ, Bjorkman D, Fennerty MB, Locke GR, Olden K, Peterson W, et al. Systematic review on the management of irritable bowel syndrome in North America. Am J Gastroenterol 2002;97(11 Suppl):S7-26.

55. Masud MA, Hasan M, Khan AK. Irritable bowel syndrome in a rural community in Bangladesh: prevalence, symptoms pattern, and health care seeking behavior. Am J Gastroenterol 2001;96(5):1547-52.

56. Veale D, Kavanagh G, Fielding JF, Fitzgerald O. Primary fibromyalgia and the irritable bowel syndrome: different expressions of a common pathogenetic process. Br J Rheumatol 1991;30(3):220-2.

57. Sperber AD, Atzmon Y, Neumann L, Weisberg I, Shalit Y, Abu-Shakrah M, et al. Fibromyalgia in the irritable bowel syndrome: studies of prevalence and clinical implications. Am J Gastroenterol 1999;94(12):3541-6.

58. Aaron LA, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. Arch Intern Med 2000;160(2):221-7.

59. Camilleri M, Heading RC, Thompson WG. Clinical perspectives, mechanisms, diagnosis and management of irritable bowel syndrome. Aliment Pharmacol Ther 2002;16(8):1407-30.

60. Boyce PM, Koloski NA, Talley NJ. Irritable bowel syndrome according to varying diagnostic criteria: are the new Rome II criteria unnecessarily restrictive for research and practice? Am J Gastroenterol 2000;95(11):3176-83.

61. Mearin F, Badia X, Balboa A, Baro E, Caldwell E, Cucala M, et al. Irritable bowel syndrome prevalence varies enormously depending on the employed diagnostic criteria: comparison of Rome II versus previous criteria in a general population. Scand J Gastroenterol 2001;36(11):1155-61.

62. Talley NJ, Gabriel SE, Harmsen WS, Zinsmeister AR, Evans RW. Medical costs in community subjects with irritable bowel syndrome. Gastroenterology 1995;109(6):1736-41.

63. Saito YA, Locke GR, Talley NJ, Zinsmeister AR, Fett SL, Melton LJ, 3rd. A comparison of the Rome and Manning criteria for case identification in epidemiological investigations of irritable bowel syndrome. Am J Gastroenterol 2000;95(10):2816-24.

64. Talley NJ, Boyce PM, Jones M. Predictors of health care seeking for irritable bowel syndrome: a population based study. Gut 1997;41(3):394-8.

65. Gomborone JE, Gorard DA, Dewsnap PA, Libby GW, Farthing MJ. Prevalence of irritable bowel syndrome in chronic fatigue. J R Coll Physicians Lond 1996;30(6):512-3.

66. Aaron LA, Buchwald D. A review of the evidence for overlap among unexplained clinical conditions. Ann Intern Med 2001;134(9 Pt 2):868-81.

67. Swiatkowski M, Rybakowski JK. Depression and T lymphocytes in patients with irritable bowel syndrome. J Affect Disord 1993;28(3):199-202.

68. Masand PS, Kaplan DS, Gupta S, Bhandary AN, Nasra GS, Kline MD, et al. Major depression and irritable bowel syndrome: is there a relationship? J Clin Psychiatry 1995;56(8):363-7.

69. Mertz H. Role of the brain and sensory pathways in gastrointestinal sensory disorders in humans. Gut 2002;51 Suppl 1:i29-33.

70. Baldwin D, Rudge S. The role of serotonin in depression and anxiety. Int Clin Psychopharmacol 1995;9 Suppl 4:41-5.

71. Kwan AC, Hu WH, Chan YK, Yeung YW, Lai TS, Yuen H. Prevalence of irritable bowel syndrome in Hong Kong. J Gastroenterol Hepatol 2002;17(11):1180-6.

72. Hu WH, Wong WM, Lam CL, Lam KF, Hui WM, Lai KC, et al. Anxiety but not depression determines health care-seeking behaviour in Chinese patients with dyspepsia and irritable bowel syndrome: a population-based study. Aliment Pharmacol Ther 2002;16(12):2081-8.

5 DISCUSSION

5.1 FINDINGS AND AIMS

The first aim of this dissertation was to examine associations for Rome II defined IBS in a U.S. population-based twin registry. The analysis supported previous studies performed in both population and clinic-based samples using earlier IBS symptom criteria. That is, previously established risk factors for IBS remained associated with Rome II defined IBS in this population-based sample. Women satisfied the Rome II IBS symptom criteria at a ratio of 2:1 compared to men. Age, BMI, mental self-rated health, and physical self-rated health demonstrated approximately null associations with Rome II IBS. Additionally, higher than expected rates of co-occurrence were obtained for IBS with MDD, CWP, and CFS-like illness. Similar to the few studies completed using the Rome II IBS criteria, our IBS prevalence was 4.7% (95% CI: 4.1, 5.4), which is lower than the prevalence of IBS documented in studies using earlier IBS symptom criteria.

The second aim of this work examined the co-occurrence of MDD and IBS, specifically investigating the possible genetic and environmental effects involved in their co-occurrence. A subset of the population-based Swedish Twin Registry was used for this purpose. Descriptive results of the IBS cases

were similar to the associations demonstrated in the first part of this dissertation. Novel to this work was the demonstration that neither environmental nor genetic influences confounded the association between MDD and IBS. This led to the conclusion that either MDD plays a causal role in the etiology of IBS or IBS plays a causal role in the etiology of MDD.

While the timing of onset between these disorders is an unresolved issue, there is plausibility that these disorders are manifestations of a common underlying pathology (i.e. somatization disorder), or that one is part of an as yet defined causal pathway that produces the other disorder. Support for these hypotheses is the influence of serotonin to both MDD and IBS independently.

In IBS, a dysfunction of the brain-gut axis has been implicated, as this dysfunction may trigger a number of inappropriate reflexes which alter gastrointestinal motility, secretion and absorption, thus causing the wide variety of symptoms associated with IBS (1). Additionally, certain subclasses of the neurotransmitter serotonin have been shown to partially mediate the postprandial colonic motor response (2) that is often associated with cramping, urgency, and diarrhea in patients with IBS (3, 4), and there is also evidence that a different serotonin subclass mediates the excitatory effects of serotonin in the human colon (5).

In MDD, there is substantial evidence supporting a role for the dysfunction of brain serotonergic systems (6). Specifically, the serotonin type 2 receptor has a disturbed function in depressed patients (7). Additionally, the serotonin type 3 receptor that is present in the brain is identical to the peripheral serotonin found in the gastrointestinal tract (7), further supporting a link between brain and gut serotonin abnormalities that may explain the higher than expected overlap of these two disorders in the population. Thus the hypotheses of either a common underlying pathology or an undetermined causal pathway between these disorders arise from the critical role that serotonin plays in the normal gut function and also in the brain-gut communication.

Both aims of this study support an association between MDD and IBS in population-based studies. Previous population-based studies of the association between MDD and IBS have been inconsistent (8-10). The Masand et al. study supported an association between MDD and IBS among those seeking some sort of healthcare. However this study was mainly descriptive and did not use acknowledged symptom criteria for IBS (9). Similarly, the Lydiard et al. study supported an association between MDD and IBS in a population-based sample, yet gastrointestinal symptoms were assessed in a structured psychiatric interview not designed to diagnose IBS (8). Additionally, the IBS definition was an “IBS-like” cluster of gastrointestinal symptoms, not corresponding to any particular symptom criteria for IBS (8).

In the population-based study by Talley et al., both IBS and MDD were evaluated using validated questionnaires and appropriate symptom criteria for IBS (Manning and Rome II). This study did not support an association between MDD and IBS, however it was limited to younger individuals, aged 26 years old, and thus only a subset of subjects had traversed the period of risk for MDD (10). Additionally, the stratified analysis based on Rome II criteria was underpowered. Our results fill a void in the literature through the use of accepted symptom-based criteria for both disorders, analyzing population-based samples, and having adequate sample sizes to be appropriately powered.

Similarly, both study populations demonstrated IBS prevalence rates between 2 and 5%. These estimates are lower than estimates of IBS prevalence using earlier symptom criteria, however prior studies using the Rome II IBS symptom criteria have documented similar lower prevalence rates. This discrepancy is most likely attributable to the ability of the Rome II criteria to identify a core group of individuals that exhibit the waxing and waning of symptoms associated with IBS (11, 12).

5.2 STRENGTHS

The largest strength of this study was the use of two population-based samples of twins. Since prior studies on the co-occurrence of IBS and MDD had been studied mainly in clinical populations, multiple biases were possible.

For example, health-care seeking bias as well as Berkson's bias has plagued these studies. The use of patient populations could induce a bias as those that actively seek care may be different than those that do not seek care for their disorders. In particular, individuals seeking treatment for IBS related symptoms evidence greater depressive symptoms than individuals with IBS who do not seek care (8, 13). Thus, the results may be biased and reflective of those individuals that are actively seeking treatment for either or both disorders. Additionally, hospital-based case-control studies have suffered from Berkson's bias as hospitalized individuals were more likely to be selected into the study as either cases or controls, and therefore do not represent a random sample from the general population. This study was not influenced by either of these biases, and included individuals with all levels of disease severity. Therefore, it provides a new viewpoint on the co-occurrence of IBS and MDD.

Both of our studies also employed the Rome II IBS symptom criteria. These are the most recent symptom criteria, and are more specific than prior criteria (12). Thus these studies allow for a better understanding of the Rome II criteria in a general population.

The analytic methods employed in these studies were additional strengths. These methods included a built-in sensitivity analysis in an attempt to address the timing of onset between IBS and MDD, a correlation between

the diagnostic algorithm for IBS used in the SALT study and the Rome II IBS definition in the AHP study in order to use similar IBS definitions in both studies, and a co-twin control study to disentangle the complex genetic and environmental influences on the co-occurrence of MDD and IBS.

5.3 LIMITATIONS

One of the main concerns of this study was its cross-sectional nature. In both studies, disorders were assessed at the same time, whether in the questionnaire or over the telephone. Thus, it was not possible in either of these studies to address the timing of onset, or causality between any of the disorders studied. In the first study, the AHP study, we attempted to address the issue of timing of onset between IBS and MDD through a sensitivity analysis. This had limited applicability, since there were only 69 participants that satisfied the criteria for both Rome II IBS and MDD. While this number was too small to fully address which disorder occurred first, the magnitude of the association was similar when the dependent and independent variables were switched. Thus while the timing of onset is still undetermined, the association between MDD and IBS is robust among individuals that fulfill IBS and MDD symptom criteria.

Additionally, there were limitations to the AHP survey that were beyond our control. Shortly after the initial mailing of the survey, all human subjects research at Virginia Commonwealth University was stopped by the U.S.

Department of Health and Human Services Office for Human Research Protections. The reason why research was halted at VCU was due to concerns of 'secondary subjects', which is relatives of the participant who are not directly participating in the study, but the participant is answering questions about their family history of disorders for close relatives (14, 15). Thus the issue became, should informed consent be obtained from the relatives as well as the study participant, thus causing the shut-down of all human studies until this ethical question was resolved. The ethicality of this study was not questioned during the shut-down, but as a result of the shut-down, our study was terminated earlier than anticipated.

Due to these events, our study was limited to early responders since only the initial mailing was sent and no follow-up mailings were completed. Additionally, it also included only those who had a correct address on file with the Mid-Atlantic Twin Registry. Another consequence of the early termination was that no telephone follow-up of non-responders was completed, nor was the planned reliability sub-study performed. Finally, no telephone diagnostic interviews or clinical evaluations were conducted to confirm the disorders.

Another limitation with the AHP data was that zygosity data were only available on a minority of subjects. This limited the usefulness of this twin dataset, as zygosity determination is a cornerstone of a twin analysis. In response to this, no twin analysis was performed on this data, but rather it

was used to obtain a better understanding of Rome II IBS, and to validate the IBS algorithm used in the SALT study.

The sampling and response to the AHP survey was another limitation. The response rate was about 30%, again related to the study being halted and due to uncertain mailing practices of the company hired to distribute the survey. The issue was that we did not know how many twins actually received the survey, and thus a response rate cannot be calculated due to the missing denominator. The estimated response rate is around 30%, and that estimate is likely the minimum response rate.

A limitation specific to the SALT study was the definition of IBS. This study included no Rome criteria, but rather applied a diagnostic algorithm to participant responses. Thus, it was not certain if the individuals identified with IBS would have been classified as having IBS based on Rome II criteria. To address this issue, a concordance of the SALT study IBS questions to the Rome II IBS questions in the AHP survey was completed. The results demonstrated that by using a subset of the SALT study IBS questions, an excellent agreement to Rome II IBS was made. This allowed for more confidence in the IBS definition from the SALT study.

Both studies had the limitation of being self-reported and not having psychiatric evaluations, clinical exams, or medical record reviews to confirm the disorders reported. Thus, all of these disorders would be subject to

misclassification. It is possible that individuals with a disorder were willing participants and thus very diligently responded to the questions, possibly leading to an overestimate. However it is also possible that individuals with a disorder were less likely to participate, which would lead to an underestimate of the number of individuals satisfying symptom criteria for these disorders. If either of these scenarios occurs, our results would likely be biased. However both studies used previously validated instruments, and the prevalence of all disorders we examined were similar to prevalences previously reported in the literature. Thus the amount of bias was probably minimal.

5.4 PUBLIC HEALTH SIGNIFICANCE

This study examined characteristics of co-occurring disorders with IBS and whether a common familial biologic or environmental mechanism underlies the co-occurrence of IBS and MDD. This study corroborates previous research demonstrating associations between IBS and MDD, CWP, and CFS. Thus more research into the etiology of these associations and overlapping co-occurrences should aid in our understanding of these disorders that affect considerable portions of the population.

Additionally, our results demonstrated that a common familial biologic or environmental mechanism was not important to the co-occurrence of IBS and MDD. Rather using the results, we can conclude that one of these disorders is causally affecting the other. Thus future research should look

further into the etiology of both disorders independently, and specifically into the timing of onset between IBS and MDD. Once the order of onset between these disorders is discovered, then future advances to the treatment of the co-occurrence of these disorders can be made.

5.5 REFERENCES

1. Houghton LA, Foster JM, Whorwell PJ. Alosetron, a 5-HT3 receptor antagonist, delays colonic transit in patients with irritable bowel syndrome and healthy volunteers. Aliment Pharmacol Ther 2000;14(6):775-82.

2. von der Ohe MR, Hanson RB, Camilleri M. Serotonergic mediation of postprandial colonic tonic and phasic responses in humans. Gut 1994;35(4):536-41.

3. Choi MG, Camilleri M, O'Brien MD, Kammer PP, Hanson RB. A pilot study of motility and tone of the left colon in patients with diarrhea due to functional disorders and dysautonomia. Am J Gastroenterol 1997;92(2):297-302.

4. Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS). Patients' description of diarrhea, constipation and symptom variation during a prospective 6-week study. Eur J Gastroenterol Hepatol 1998;10(5):415-21.

5. Borman RA, Tilford NS, Harmer DW, Day N, Ellis ES, Sheldrick RL, et al. 5-HT(2B) receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro. Br J Pharmacol 2002;135(5):1144-51.

6. Lerer B, Macciardi F, Segman RH, Adolfsson R, Blackwood D, Blairy S, et al. Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 2001;6(5):579-85.

7. Leonard BE. Fundamentals of Psychopharmacology. 2nd Edition ed. New York, NY: John Wiley & Sons; 1997.

8. Lydiard RB. Irritable bowel syndrome, anxiety, and depression: what are the links? J Clin Psychiatry 2001;62 Suppl 8:38-45; discussion 46-7.

9. Masand PS, Kaplan DS, Gupta S, Bhandary AN, Nasra GS, Kline MD, et al. Major depression and irritable bowel syndrome: is there a relationship? J Clin Psychiatry 1995;56(8):363-7.

10. Talley NJ, Howell S, Poulton R. The irritable bowel syndrome and psychiatric disorders in the community: is there a link? Am J Gastroenterol 2001;96(4):1072-9.

11. Boyce PM, Koloski NA, Talley NJ. Irritable bowel syndrome according to varying diagnostic criteria: are the new Rome II criteria unnecessarily restrictive for research and practice? Am J Gastroenterol 2000;95(11):3176-83.

12. Camilleri M, Heading RC, Thompson WG. Clinical perspectives, mechanisms, diagnosis and management of irritable bowel syndrome. Aliment Pharmacol Ther 2002;16(8):1407-30.

13. Blanchard EB, Scharff L, Schwarz SP, Suls JM, Barlow DH. The role of anxiety and depression in the irritable bowel syndrome. Behav Res Ther 1990;28(5):401-5.

14. Botkin J. Protecting the privacy of family members in survey and pedigree research. Jama 2001;285(2):207-11.

15. Mathews J. Father's Complaints Shut Down Research; U.S. Agencies Act On Privacy Concerns. The Washington Post 2000;Sect. B.07.