Irritable bowel syndrome

- a disorder of gut-brain interaction

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To the kids and mom; you're the best!

-Don't worry.

Jesus

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ABSTRACT

Irritable bowel syndrome (IBS) is a common and multifactorial functional gastrointestinal (GI) disorder characterized by altered gut-brain communication. Due to the complexity of gut-brain interactions, the aim of this thesis was to enhance the understanding of associations between GI symptom severity and measures from multiple levels along the gut-brain axis in IBS patients.

In this thesis, various indications of altered gut-brain interactions were demonstrated: I. IBS patients with anxiety or depression reported more severe GI and non-GI symptoms than patients without psychological distress. Visceral hypersensitivity, aberrant function of the autonomic nervous system, GI-specific anxiety, and non-GI somatic symptoms differentiated between patients with and without psychological distress. II. Overall, modest associations were discovered among neurophysiological factors, and between neurophysiological factors and the severity of IBS symptoms. The most important combination of neurophysiology measures for GI symptom severity in IBS patients were extracted through a computerized method and were found to be visceral hypersensitivity and psychological distress. III. Alterations in a wide range of psychological measures were common in IBS. A strong cumulative effect of psychological alterations on the severity of GI symptoms was found. IV. Central sensitization was frequent in IBS patients, but the severity of central sensitization and GI symptoms were only modestly associated in IBS, suggesting that the presence, rather than the level, of central sensitization is of importance for GI symptoms in IBS.

In conclusion, the results from this thesis support the current view of IBS being a disorder of gut-brain interaction, where both peripheral and central factors contribute to this multifactorial disease. Complex associations between psychological and neurophysiology measures, and the severity of GI symptoms were demonstrated in the studies included in this thesis.

Keywords: Irritable Bowel Syndrome, gastrointestinal symptom severity, anxiety, depression, neurophysiological aberrations, psychological alterations, central sensitization.

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SAMMANFATTNING PÅ SVENSKA

Irritable bowel syndrome (IBS) är en funktionell mag-tarmsjukdom som karaktäriseras av kroniska eller återkommande buksmärtor och avföringsrubbning, utan att det finns synliga förändringar i tarmen. Ungefär 5 % av befolkningen rapporterar symtom förenliga med IBS. I tillägg till mag-tarmsymtomen rapporterar många IBS-patienter psykiska besvär och symtom utanför mag-tarmkanalen. Orsakerna till sjukdomen är oklara, men avvikande samspel mellan nervsystemet i tarmen och i hjärnan anses vara av betydelse. I denna avhandling analyseras sambanden mellan svårighetsgrad av magtarmsymtom, exempelvis buksmärta, buksvullnad, förstoppning och diarré, och andra symtom, samt olika mått på nervsystemets funktion. I det första manuskriptet fokuserar vi på betydelsen av ångest och depression vid IBS; i det andra manuskriptet på avvikelser i tarmens nervsystem, hjärnan, och det icke viljestyrda nervsystemet; i det tredje manuskriptet på samspelet mellan olika psykologiska avvikelser och dess betydelse för mag-tarmsymtomen vid IBS; och i det fjärde manuskriptet på central sensitisering, som är en ökad känslighet för smärta, obehag, och externa intryck, såsom starka ljus, höga ljud, dofter och kemikalier, och dess betydelse för mag-tarmsymtom. Det övergripande målet med avhandlingen är att öka kunskapen om sjukdomen och om mekanismerna bakom symtomen, vilket i sin tur kan leda till en bättre, individanpassad behandlingsplan för IBS-patienter.

I det första manuskriptet påvisade vi att ångest och depression är vanligt vid IBS och associerat med svårare magtarmsymtom och andra kroppsliga symtom.

I det andra manuskriptet identifierade vi att avvikelser i nervsystemet samverkar med mag-tarmsymtom hos IBS-patienter. De viktigaste komponenterna för symtom vid IBS var en ökad smärtkänslighet i tarmen och psykologiska faktorer.

I det tredje manuskriptet påvisade vi att svårighetsgraden av mag-tarmsymtom vid IBS påverkas starkt av antalet och svårighetgraden av olika psykologiska avvikelser, såsom stress, trötthet, ångest och hantering av smärta.

I det fjärde manuskriptet beskrev vi att central sensitisering är ett vanligt fenomen vid IBS och att detta är kopplat till svårighetsgraden av mag-tarmsymtom.

Sammanfattningsvis understryker resultaten från denna avhandling betydelsen av interaktioner mellan tarm och hjärna för mag-tarmsymtom vid IBS, vilket bör tas i beaktande vid behandling av dessa patienter.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Midenfjord, I, Polster, A, Sjövall, H, Törnblom, H, Simrén, M. *Anxiety and depression in irritable bowel syndrome: Exploring the interaction with other symptoms and pathophysiology using multivariate analyses.* Neurogastroenterol Motil. 2019; 31: e13619.

II. Midenfjord, I, Polster, A, Sjövall, H, Friberg, P, Törnblom, H, Simrén, M. *Associations among neurophysiology measures in irritable bowel syndrome (IBS) and their relevance for IBS symptoms.* Sci Rep. 2020;10:9794.

III. Midenfjord, I, Borg, A, Törnblom, T, Simrén, M. *Cumulative effects of psychological alterations on gastrointestinal symptoms in irritable bowel syndrome.* Am J Gastroenterol, 2020 Nov 4, Online ahead of print.

IV. Midenfjord, I, Grinsvall, C, Koj, P, Carnerup, I, Törnblom, H, Simrén, M. *Central sensitization and severity of gastrointestinal symptoms in irritable bowel syndrome, chronic pain syndromes and inflammatory bowel disease.* In manuscript.

CONTENT

ABBREVIATIONS

ANS	Autonomic Nervous System
CNS	Central Nervous System
CSI	Central Sensitization Inventory
GAD-7	Generalized Anxiety Disorder, 7-item scale
GI	Gastrointestinal
GSRS-IBS	Gastrointestinal Symptom Rating Scale, IBS version
HAD	Hospital Anxiety and Depression scale
HAD-A	HAD, Anxiety domain
HAD-D	HAD, Depression domain
HSP	Highly Sensitive Person scale
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IBS-C	IBS with predominant constipation
IBS-D	IBS with predominant diarrhea
IBS-M	IBS with mixed bowel habits
IBS-SSS	IBS Severity Scoring System
IBS-U	IBS unclassified
Lasso	Least Absolute Selection and Shrinkage Operator regression
PHQ-9	Patient Health Questionnaire-9

DEFINITIONS IN SHORT

Neurophysiology	The physiology of the nervous system.[1]
Pathophysiology	The physiology of abnormal states.[1]
Rome criteria	Diagnostic criteria for functional gastrointestinal disorders.
Partial η^2	A measure of the impact of a linear trend analysis.
z-score	A number standardized by the group mean.
Spearman's ρ	A non-parametrical correlation coefficient.
Linear trend	A correlation analysis between group means.
Principal component analysis	A multivariate analysis method exploring the possible differences between groups.
Orthogonal Partial Least Squares-Discriminant Analysis	A multivariate analysis method extracting the most important differentiating factors between groups.
Test-retest	A test of the consistency in the scoring of a questionnaire between two time points.
Cronbach's alpha	The averaged consistency of a questionnaire between two time points.
Intraclass correlation coefficient	The consistency of each individual question in a questionnaire in each study participant, measured between two time points.

1 INTRODUCTION

1.1 IRRITABLE BOWEL SYNDROME (IBS)

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder.[2] It is characterized by recurrent or chronic abdominal pain, which is associated with defecation and related to changes in frequency and form of stool. IBS is a female predominant disorder,[3,4] affecting around 5% of the general population,[5] and is more common in individuals under the age of 50.[4,6] IBS does not shorten the expected life span,[7] but the symptoms are experienced as disabling, bothersome, painful, and embarrassing,[8] negatively affecting quality of life,[9] as well as work productivity.[8]

The diagnosis is based on a careful clinical history, including the presence of current symptoms and a disease duration of at least 6 months. The diagnostic symptoms, i.e. abdominal pain in combination with altered bowel habits, must be present on average at least one day a week.[10] In addition to these functional abdominal symptoms, absence of alarm symptoms, such as rectal bleeding, unintended weight loss or nocturnal symptoms, should be confirmed. Based on the clinical symptom profile, a limited number of tests might be considered in certain patients, excluding bile salt malabsorption, celiac disease, thyroid malfunction and intestinal inflammation.[11] The Rome IV diagnostic criteria questionnaire is a comprehensive diagnostic questionnaire assessing symptoms characteristic of functional GI disorders and might be used to confirm the diagnosis (**Table 1**).[10]

1.2 DIAGNOSTIC CRITERIA AND EPIDEMIOLOGY

The diagnostic criteria for IBS have evolved during the last twenty years, as seen in **Table 1**. In this thesis, the second to fourth versions of the Rome criteria are used for diagnosing IBS. The most important changes between the second and third Rome criteria is the duration of the symptoms. In Rome II, the criteria consists of having GI symptoms during a minimum of twelve non-consecutive weeks in the past twelve months,[12] whereas in Rome III, the GI symptoms must have been present at least three days per month in the last three months.[13] In Rome IV,[2] the criteria of the presence of abdominal pain or discomfort, described in Rome II[12] and Rome III,[13] was changed to only presence of abdominal pain.

Table 1: The Rome Criteria for IBS.

Version	Criteria
Rome II[12]	Twelve non-consecutive weeks in the preceding 12 months of abdominal discomfort or pain that has two out of three features: • Relieved with defecation; and/or • Onset associated with a change in frequency of stool; and/or • Onset associated with a change in form (appearance) of stool
Rome III[13]	Recurrent abdominal pain or discomfort, 3 days per month in the last 3 months (12 weeks), associated with ≥2 of the criteria below: • Improvement with defecation • Onset associated with a change in stool frequency • Onset associated with a change in stool form (appearance) The criteria are fulfilled with symptom onset 6 months prior to diagnosis.
Rome IV[2]	Recurrent abdominal pain, on average at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria: • Related to defecation • Associated with a change in frequency of stool • Associated with a change in form (appearance) of stool Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.

This change was based on the different understanding and definition of the word 'discomfort' in different regions of the world. The differences between the different versions of the diagnostic criteria have affected the prevalence of IBS. The prevalence of IBS in the general population has been reported to range from 15% in Rome II,[12] to 11% in Rome III[13] and 4-5% in Rome IV.[5,14]

1.3 IBS SUBGROUPS

IBS is divided into subgroups based on the predominant bowel habit.[2] The four subgroups consist of IBS with constipation predominance (IBS-C), IBS with diarrhea predominance (IBS-D), IBS with mixed bowel pattern (IBS-M) and IBS unclassified (IBS-U), and are based on the dominating stool form described by the Bristol stool form (BSF) scale[15] (**Table 2**). Four of the seven stool form types are considered as abnormal. BSF types 1 and 2 are recognized as constipation and types 6 and 7 as diarrhea.

Table 2: The Bristol stool form scale.[15]

Bristol stool form	Description
1	separate hard lumps like nuts
2	sausage shaped but lumpy
3	like a sausage or snake but with cracks on its surface
4	like a sausage or snake, smooth and soft
5	soft blobs with clear cut edges
6	fluffy pieces with ragged edges, a mushy stool
7	watery, no solid pieces

Description of seven forms of stool, used for subgrouping of IBS patients. Forms 1, 2, 6, and 7 are regarded as abnormal.

The subgrouping in the Rome IV criteria is defined by the appearance of the stools on days with at least one abnormal stool, recorded in a daily diary of at least 14 consecutive days[2] (**Table 3**). The classification into subgroups have been slightly different between Rome diagnostic criteria versions.[2,12,13] Rome II subdivide IBS into three parts: constipation-predominant IBS, diarrhea-predominant IBS and alternating IBS,[12] whereas Rome III criteria has four subgroups,[13] fairly similar to the Rome IV subgroups.[2]

Table 3: Subgrouping of IBS according to Rome IV criteria.[2]

Subgroup	BSF type 1 or 2	BSF type 6 or 7
IBS-C	>25%	<25%
IBS-D	<25%	>25%
IBS-M	>25%	>25%
IBS-U	<25%	<25%

Subgrouping of IBS based on the number of stools on days with at least one abnormal stool, recorded in a daily diary of at least 14 consecutive days.

1.4 DISEASE BURDEN

IBS has a large economic impact both at the individual and the community level.[16,17] Many IBS patients seek health care frequently[14] and have lowered work productivity due to their GI symptoms, ranging from lowered presenteeism to full work absenteeism.[8,9] Furthermore, IBS patients often experience fatigue,[18] and reduced health-related quality of life[19,20] compared

with the general population, as well as compared with patients suffering from many other diseases. The disorder affects the patient's ability to maintain social activities, influences their parenting abilities and disturbs the development and upholding of close relationships.[19] In IBS patients with somatic and/or psychological comorbidities, the quality of life is lower and the GI symptoms are more severe compared with IBS patients without these comorbidities.[21] Examples of common comorbidities or extraintestinal symptoms in IBS are anxiety, depression, somatization, migraine headaches, fibromyalgia, interstitial cystitis, and chronic fatigue.[2,22]

1.5 IBS MANAGEMENT

The management of the IBS patient is symptom-based and has a stepwise approach (**Figure 1**). The foundation of the management plan is a good physician-patient relationship, which facilitates the communication of the diagnosis and the treatment plan, explaining and reassuring the patient of the benign nature of IBS, and communicating life style advice, including decreased intake of alcohol, caffeine, fat and spicy foods.[11,23]

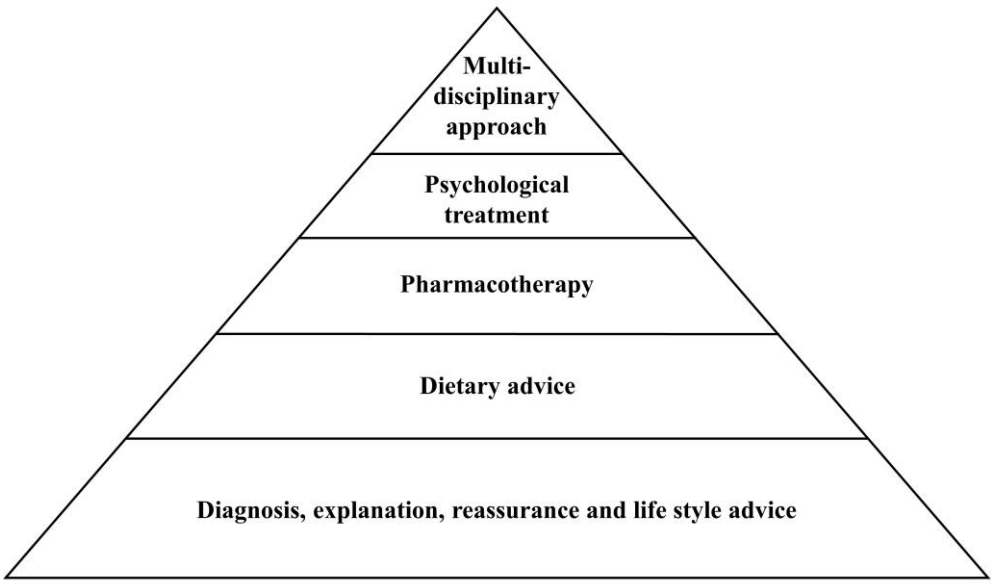

Figure 1. Stepwise management approach of IBS patients.

Patient education about IBS, consisting of verbal information during the outpatient visit, informative leaflets and in some cases, more extensive patient information given in groups or over the internet is also central. For many IBS

patients, this basic management step is sufficient, and no further treatment is necessary. For others, an individualized management plan focusing on the main symptom is applied. General dietary advice is of value to a large proportion of patients. Smaller portion sizes, eating more often, chew the food thoroughly, and not stress during meals are general factors considered of importance. A proportion of patients benefit from adjustment of the dietary fiber content in the food[24] or the addition of probiotics.[25] Others benefit from elimination and thereafter careful reintroduction of a group of fermentable carbohydrates, i.e. fermentable oligosaccharides, disaccharides, monosaccharides and polyols (the low FODMAP diet).[26] In patients with severe or persisting symptoms, pharmacotherapies targeting the main symptom, consisting of antidiarrheal drugs,[11] laxatives,[11,27,28] or drugs directed towards abdominal pain,[27] or bloating[23,28] are commonly used. Psychological treatment options, i.e. cognitive behavioral therapy, psychodynamic psychotherapy, mindfulness or gut-directed hypnotherapy, are used as the next management step in subsets of IBS patients.[11] In patients with severe and refractory IBS, a multidisciplinary treatment approach involving different types of health care professions can be advocated, potentially also including augmentation therapy that combines two or more therapeutic options for added effects, such as pharmacotherapy with psychotropic drugs and psychological treatment.[11,29]

However, even if this management approach is used and all the different levels are tested, a proportion of IBS patients still have persisting symptoms. This supports an incomplete understanding of the pathophysiology in IBS and its association to symptoms. Therefore, this thesis addresses multiple symptoms and neurophysiological alterations, and their complex association to the severity and pattern of GI symptoms in IBS, in order help to optimize the future management of this large patient group.

1.6 PATHOPHYSIOLOGY OF IBS: A DISORDER OF GUT-BRAIN INTERACTION

The pathophysiology of IBS is complex and incompletely known. Factors of importance for the development of IBS are both of innate and acquired nature (**Figure 2**). Today IBS and other functional GI disorders are viewed as disorders of altered gut-brain interactions. This view is supported by studies demonstrating abnormalities in the function of the autonomic and central nervous systems (ANS, CNS), indicated by abnormalities in ANS measures, such as the function of the baroreceptors.[30] and in direct and proxy measures of CNS function, such as CNS activation,[31–33] central sensitization[34–39] and the absence or presence of anxiety, and depression.[22,40,41] Altered intestinal

function such as abnormal intestinal motility,[34,42–47] altered GI secretion,[48,49] heightened permeability,[50] and visceral hypersensitivity[34,38,51] are also common among IBS patients. Other alterations, outside of the focus of this thesis, but previously described in IBS, are changes seen in the function of the immune system,[52] in the composition of the microbiota,[50,53] and in genetic[54] and inflammatory markers.[55]

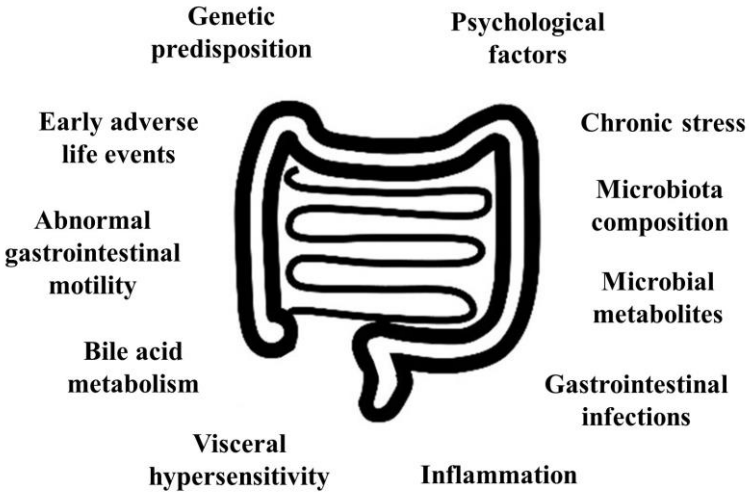

Figure 2. Key pathophysiological factors in IBS.

Most previous studies in IBS patients have explored one or a few of these pathophysiological alterations at a time, but in the studies included in this thesis the aim was to focus on simultaneous analysis of multiple factors in order to increase the knowledge of the gut-brain interactions in IBS. The factors analyzed in this thesis are described in greater detail below.

1.6.1 GUT-BRAIN INTERACTIONS

The gut and the brain communicate through the autonomic nervous system (subdivided into the sympathetic, parasympathetic and enteric nervous systems), hormones, and immune cells.[56,57] Visceral afferent pathways (enteric, spinal, and vagal) transmits information from the gut to the central nervous system (CNS).[58] Within the gut, enteric nervous system (ENS) reflex circuits upholds the basal homeostasis through regulation of motility, secretion, and blood flow, and descending corticolimbic pathways regulates and modulates the gut function through influencing the responsiveness of the

ENS reflexes (**Figure 3**). Through an intrinsic system of regulatory loops in the descending pathways, only a small proportion of the signals from the gut reaches the conscious level as sensation or pain in the healthy state.[56,58]

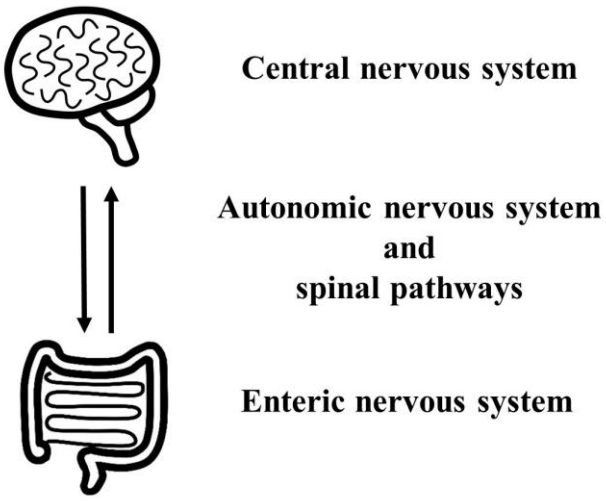

Figure 3. Schematic overview of the central, autonomic and enteric nervous systems.

In IBS, small, but distinguishable, alterations are seen at multiple sites of the gut-brain axis.[58–60] Alterations along the gut-brain axis are seen in subsets of IBS patients, including abnormal microbiota composition,[61] gut permeability,[58,62,63] and immune function,[50,63] as well as low grade inflammation,[50,52] and altered ANS[60,64,65] and CNS function.[22,66–69] Furthermore, GI symptoms in IBS are found to be more severe when high levels of stress,[70,71] fatigue,[18,72] and other comorbidities[22] are present. The communication in the gut-brain axis is bidirectional and complex, and due to its complexity, the gut-brain interactions in IBS are not yet fully elucidated. In this thesis, measures from multiple sites along the gut-brain axis are simultaneously analyzed to gain more knowledge of the altered gut-brain interactions in IBS.

1.6.2 CENTRAL NERVOUS SYSTEM ALTERATIONS

Structural and functional brain alterations. Structural and functional changes have been seen in certain brain areas in IBS patients. Changes in gray matter density have been seen in parts of the corticolimbic system in IBS, which regulates learning and memory, but also in areas involved in stress and arousal.[32,67] Although certain structural changes have been seen, some of these associations were not significant after controlling for anxiety and depression,[31] which indicate that psychological comorbidity has a significant role in the

structural changes seen in IBS patients. Moreover, different activation patterns are seen in IBS patients in comparison with healthy controls during visceral stimulation,[73] in areas associated with interoception, salience and sensory processing,[33] as well as in somatosensory, cognitive, and affective processing.[35]

Central sensitization. Central sensitization is described as heightened CNS response to peripheral signals.[74] This continuous state of heightened sensitivity, common in IBS, is expressed through hyperalgesia, an exaggerated and prolonged response to noxious stimuli, allodynia, a feeling of pain from normally innocuous stimuli,[75,76] and hypersensitivity to sensory input, such as noise, bright light and chemical substances.[77,78] Somatization is considered as a component of the wider concept of central sensitization,[79,80] and assessment of somatic symptoms is a part of the evaluation of central sensitization in the questionnaires used in this thesis.[81,82] Central sensitization is theorized to be one of the significant mechanisms behind the central sensitization syndromes,[75,83] a group of functional disorders with non-organic pain lasting for more than three months. This group consists of e.g. IBS, fibromyalgia, chronic low back pain, chronic tension headache and temporomandibular joint disorder.[84] These disorders are commonly co-occurring.[85–88] Signs of central sensitization, such as rectal[34–37] and somatic[35,38] hyperalgesia and allodynia, are commonly found in IBS, and enhanced rectal sensitivity is associated with the severity of GI symptoms in IBS.[37] Central sensitization in the form of sensitivity for sensory input, expressed by heightened perception of bright lights, smells, caffeine and other chemicals, have not yet been extensively studied in IBS. Only one study has so far explored sensory input sensitivity in IBS patients, finding implications of hypersensitivity for chemicals and sound in the disorder.[39] Individual measures of central sensitization have previously been assessed in IBS, but these studies were limited as they only included a small part of the full range of central sensitization symptoms and measures. Therefore, all three definitions of central sensitization were combined and analyzed in Manuscript IV, to elucidate the importance of central sensitization in IBS and for GI symptoms.

Psychological factors. Psychological factors, such as self-reported anxiety, depression, GI-specific anxiety, and somatization, are suggestive of altered CNS function and are often used as proxy measures of CNS dysfunction.[89–91] Psychological distress, here defined as the presence of anxiety or depression, is common in IBS.[22,40,41,68] Patients with concurrent anxiety and depression, have more severe symptoms from the GI tract,[92–95] and visceral hyper-sensitivity is common.[37,96–98] Psychological distress in IBS is associated with the severity of somatic symptoms,[69,99] lower quality of life,[95,100] and alterations

in ANS function,[101] colonic motility,[102,103] and immune function,[104,105] as well as changes in the composition of gut flora.[106,107] Psychological distress has been shown to precede IBS in one study,[108] but more extensive longitudinal studies are warranted before the causality of anxiety and depression to IBS can be determined. IBS patients are commonly affected by general anxiety, but also with GI-specific anxiety, i.e. anxiety for symptoms from the GI tract.[109,110] This specific anxiety is triggered by the fluctuating and unpredictable symptoms associated with the disorder. It is related to the severity of GI symptoms, as well as with general anxiety and depression.[109] Somatization, or non-GI somatic symptoms, is more common in IBS patients than in the general population[111] and is associated with the severity of GI symptoms.[99,112,113] In this thesis, psychological factors were analyzed in combination with other factors considered to be of importance for IBS, to further elucidate their role for GI symptoms in IBS.

1.6.3 AUTONOMIC NERVOUS SYSTEM ALTERATIONS

Autonomic nervous system (ANS) alterations have been demonstrated in subsets of IBS patients, with subtle differences in heart rate variability (HRV) and baroreceptor function. These two measures are two of the most common ways to measure the function of the ANS in IBS. Studies of HRV, i.e. the difference in length between consecutive heartbeats, in IBS have revealed alterations in the balance between parasympathetic and sympathetic activity, shown as aberrant HRV frequency and time measures.[65,114,115] These altered measures of ANS processing suggest impairment of vagal regulation.[114] Also, connections between HRV alterations and perceived stress and somatic symptoms have been demonstrated in IBS.[64] Regarding differences between subgroups, more distinct changes in HRV measures have been noticed in patients with IBS-C compared to patients with IBS-D.[65] Moreover, lowered parasympathetic activity has been observed in IBS patients with a history of psychiatric diseases than in non-psychologically distressed IBS patients.[101] Baroreceptor sensitivity, another measure of ANS function, was detected to be lower in IBS patients in rest compared with healthy controls.[30] This measure was significantly higher before a test of visceral sensitivity in IBS patients compared with volunteers,[116] which might reflect anxiety for rectal distensions.[92] ANS alterations were explored using multivariable and multivariate analysis in this thesis, exploring their relevance for the characterization of IBS patients with psychological distress, and for GI symptom severity in IBS, including interactions with other neurophysiology measures.

1.6.4 VISCERAL HYPERSENSITIVITY

Visceral hypersensitivity, now regarded as one of the hallmarks of IBS, is found in around half of IBS patients.[34,38,51] It is defined by a decreased pain threshold for visceral distensions or enhanced intensity of visceral sensations.[117] Visceral hypersensitivity has shown associations with the severity of GI symptoms,[37,93] somatization,[118] younger age,[98,118] and, in some studies, with IBS subtype,[51] but diverging associations have been found with psychological distress.[96,118] IBS patients with visceral hypersensitivity have been distinguished from normosensitive patients by reporting more severe abdominal pain and bloating.[119,120] Furthermore, IBS patients with visceral hypersensitivity also have shown higher cutaneous thermal sensitivity and larger pain inhibition deficits than normosensitive IBS patients.[121] Moreover, IBS patients also exhibit alterations in the functional responsiveness and connectivity of the brain,[33,67] as well as greater neural activity[35] during visceral stimuli compared with healthy volunteers. Few studies have analyzed visceral sensitivity simultaneously with other factors. Therefore, visceral hypersensitivity was included as one of the factors of the multivariable and multivariate analysis in this thesis, in order to elucidate its role for GI symptoms in IBS.

1.6.5 ALTERED INTESTINAL SECRETOMOTOR FUNCTION

Colorectal motility. The motility of the large intestine has gained attention in IBS, as subsets of IBS patients have shown accelerated or delayed colonic transit.[34,42–45] Gender differences in motility[42,43] and associations to the GI symptom pattern[34,42,102] have been seen, but with partly discordant results. In general, accelerated colonic transit has been associated with loose, frequent stools, whereas delayed transit was primarily seen in IBS patients with hard and infrequent stools.[42–45] An association between number of days within the study period with abnormal number of bowel movements and colonic transit time has also been observed.[102] Furthermore, altered rectal compliance and tone have been detected in subsets of IBS patients, but with no obvious association with the symptom pattern.[51,122–125]

Small intestinal motility. The motility of the small intestine in IBS has primarily been described in small studies. Alterations have been demonstrated in the motility pattern of the small intestine, but larger studies are needed to confirm the relevance of these findings. During day time measurement, the frequency of the migrating myoelectric complexes was higher in IBS than in volunteers in one study.[126] In another study, an increased number of retrograde

pressure waves was seen in IBS-D patients when measuring the fasting and fed intestinal propagation patterns with high-resolution manometry equipment.[46] In the same study, a connection between increased frequency of retrograde pressure waves and GI symptoms was found. Moreover, abdominal symptoms of discomfort or distension have been described to follow certain motor patterns in the small intestine of IBS patients.[47]

Small intestinal secretion. Secretory dysfunction in the small intestine has been observed in subsets of IBS patients, and has been proposed to be of relevance for abnormal bowel habits in IBS.[127] In the small intestinal epithelium, active transport of chloride via the cystic fibrosis transmembrane conductance regulator (CFTR) channel is the major pathway for secretion, with water following passively paracellularly through the tight junctions.[128] The CFTR pathway can be regulated neurally, via peptide receptors, and changes in intracellular cyclic adenosine monophosphate concentration. Opening of CFTR is associated with an increased transmucosal potential difference, making it a frequently used surrogate marker for active chloride secretion. One previous study demonstrated elevated potential differences in subsets of IBS patients, mainly in IBS-D, indicative of altered small intestinal secretory function.[48] However, studies exploring the secretory function in IBS are rare.

These measures of intestinal secretomotor function have all been assessed previously in IBS, but they have not been analyzed simultaneously, nor analyzed together with other pathophysiological factors in IBS. In this thesis, these measures were therefore analyzed with each other and with various pathophysiological measures of interest, to gain a deeper understanding of their role for GI symptoms in IBS.

1.6.6 ALTERED GUT MICROENVIRONMENT

Microbiota and diet. The gut microbiota is important for gut homeostasis. It is relatively stable in healthy subjects, in contrast to the more unstable composition of the gut microbiota seen in IBS.[50,53] A diminished richness of different species in the gut microbiota of IBS patients has been detected.[53,107] This lowered abundance of species is thought to possibly influence the intestinal barrier, the immune and enteroendocrine systems, and potentially lead to GI symptoms.[50] Furthermore, certain microbiota signatures found in IBS patients have been associated with colon transit and depression,[106] and with the severity of GI symptoms.[129] Moreover, in many IBS patients, GI symptoms increase after ingestion of certain food products, such as beans,

cabbage, onions or foods rich in poorly absorbed short-chained sugars, i.e. oligosaccharides, disaccharides, monosaccharides and polyols.[130,131] These foods might increase the luminal water content in the small intestine through an osmotic effect, or the gas production in the colon through fermentation by gut bacteria, and lead to luminal distension and thereby an increase in GI symptoms.

Barrier function and altered gut permeability in IBS. The epithelium of the gut consists of a single cell layer forming a barrier against the intestinal content, simultaneously permitting water, electrolytes and nutrients to cross the barrier into the blood stream.[132] In IBS, subsets of patients show increased gut permeability, which may be caused by alterations in expression of epithelial tight junctions and adhesion molecules leading to a dysfunctional mucosal epithelium.[50] The altered barrier function of the epithelium in these patients may cause mucosal immune activation, leading to release of immune mediators threatening the barrier function of the epithelium and modifying nerve signaling within the ENS.[133]

Immunity (innate and adaptive). Heightened innate immune activation is found in subsets of IBS patients, together with indications of an activated adaptive immune system.[52] Mast cells and monocytes have been demonstrated to be of importance for IBS,[52,53,133] and altered B-cell activity and antibody production, as well as an increased number of T cells, have been seen in groups of IBS patients.[52,133,134] Furthermore, subsets of IBS patients have displayed low-grade inflammation of the epithelium,[52,135,136] abnormal neuroimmune interactions,[52] and neurohormonal alterations.[67]

2 AIM

The general aim of this thesis was to explore gut-brain interactions in IBS, specifically the associations between GI symptom severity and various pathophysiological alterations of proposed relevance in IBS. Neurophysiological examinations and questionnaires for patient-reported outcomes were used as tools to investigate the symptom burden and pathophysiological alterations in the study participants. Specific aims of the four manuscripts are listed below.

I: To characterize and to assess the symptom severity in IBS patients with anxiety and depression, and to determine the most relevant pathophysiological factors for patients with and without psychological distress.

II: To identify the most relevant neurophysiology measures for GI symptom severity in IBS, and to explore the interactions among them.

III: To explore the possible cumulative effect of various psychological alterations on the severity of GI symptoms in IBS.

IV: To investigate associations between presence of and level of central sensitization and the severity of GI symptoms in IBS patients compared with chronic pain and inflammatory bowel disease patients, and volunteers without chronic diseases.

3 PATIENTS AND METHODS

All IBS patients included in this thesis were recruited for studies evaluating the importance of various pathophysiological, neurophysiological, and psychological factors for symptoms in IBS. They received verbal and written information about the study, and signed an informed consent form prior to the study start. All studies were conducted in accordance with the declaration of Helsinki and were ethically approved by the Regional Ethical Review Board in Gothenburg. The data analysis methods, questionnaires and neurophysiological examinations used in the four manuscripts are summarized in **Figure 4** and described below.

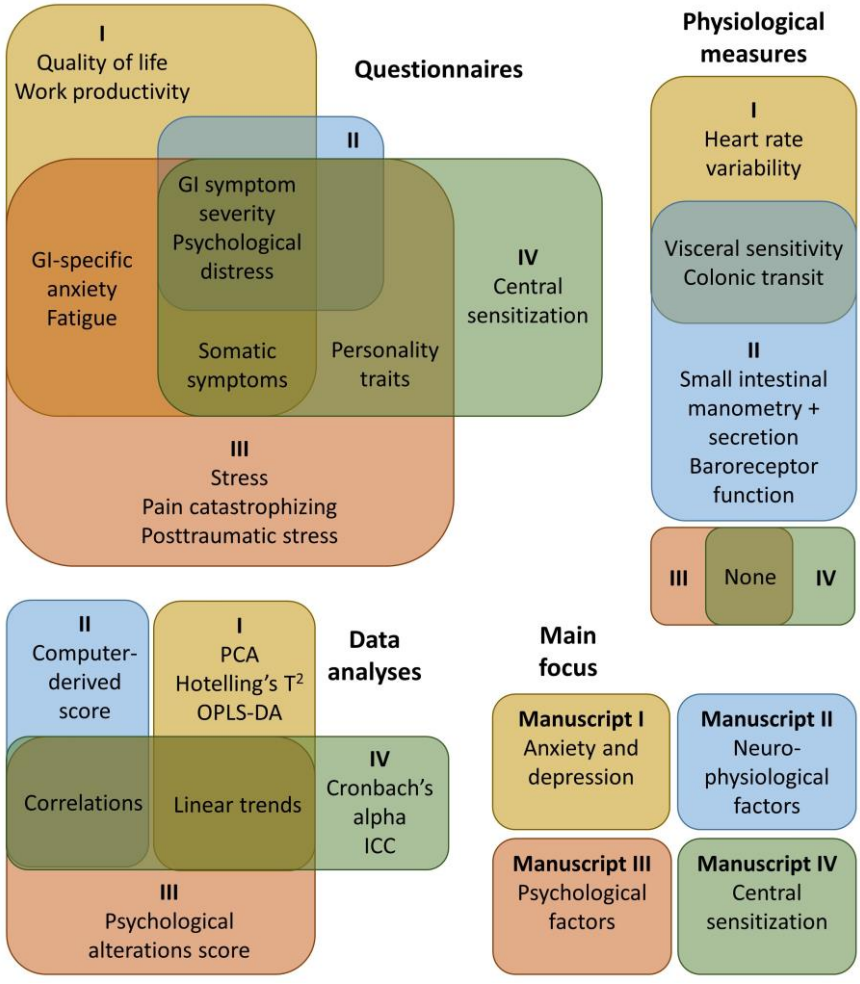

Figure 4: Overview of the four manuscripts, the measures analyzed, and the data analysis techniques used in this thesis.

3.1 CHARACTERIZATION OF THE IBS COHORTS

Four different IBS cohorts from four different time periods were analyzed in the manuscripts of this thesis (**Table 4**). The inclusion criteria for the four different IBS cohorts were to a large extent similar between manuscripts. In all manuscripts, the participants were 18–65 years at inclusion. Only IBS patients were included (with the exception of Manuscript IV), and no other GI disease explaining the GI symptoms in the IBS patients was allowed. Pregnancy or breast-feeding, as well as severe diseases were exclusion criteria in all of the manuscripts. Study participants were included according to the Rome II criteria for the diagnosis of IBS in Manuscript II, whereas the Rome III criteria were utilized in Manuscript I. Manuscripts III and IV applied the latest diagnostic version (Rome IV criteria) for the inclusion of IBS patients.

The severity of GI symptoms in the patient cohorts were moderate to severe in all manuscripts, around 70% were females, and the median age was around 35 years. In the three first manuscripts, a similar distribution of IBS subgroups appeared. Approximately a third had constipation predominant IBS, a third had diarrhea predominant IBS and a third had alternating bowel pattern. No formal subgrouping was used in Manuscript IV. There was a large overlap of IBS patients between Manuscripts I and II, and a minor overlap between patients included in Manuscripts III and IV (**Figure 5)**. Manuscripts I, II and III consisted of only IBS patients. In Manuscript IV, groups of patients with inflammatory bowel disease (IBD) (Crohn's disease and ulcerative colitis) and chronic pain diseases, as well as volunteers without documented or self-reported chronic diseases were also included for comparison with the IBS patients. The patients with chronic pain and IBD were slightly older, and the volunteers younger, than the IBS group. In comparison with the IBS group, there were more females in the chronic pain group, whereas the other groups had a lower proportion of women.

Table 4. Characteristics of the four cohorts.

	Manuscript I	Manuscript II	Manuscript III	Manuscript IV
Subjects	IBS patients	IBS patients	IBS patients	IBS patients Chronic pain patients IBD patients Volunteers
Inclusion period (years)	2006-2010, 2010-2015	2002-2007	2015-2019	2018-2020
Rome criteria	III	II	IV	IV
Number of subjects (N)	769 in total; PCA* on patho-physiological measures: 143; PCA* on symptom reporting: 404	281	106	403 in total; Test-retest: 66 (IBS: 44, volunteers: 22); Linear trends: 403 (IBS: 215, Chronic pain: 36, IBD: 40, Volunteers: 112)
Female gender (%)	73%	74%	68%	IBS: 81% Chronic pain: 92% IBD: 53% Volunteers: 59%
Age (median, interquartile range)	36 [27-48]	36 [28-50]	35 [26-45]	IBS: 32 [26-45] Chronic pain: 44 [37-53] IBD: 39 [33-53] Volunteers: 25 [22-30]
Subgroups IBS-C, IBS-D, IBS-A or IBS-M/U (%)	26%, 39%, 35%	25%, 43%, 32%	35%, 37%, 28%	-

**Principal Component Analysis.*

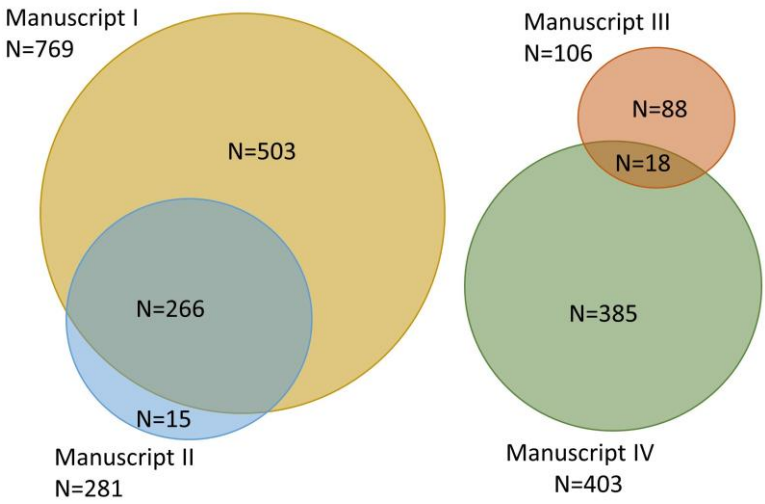

Figure 5. The overlap between patients in the cohorts in this thesis.

3.2 QUESTIONNAIRES

Questionnaires were used in all manuscripts of this thesis to assess the severity of GI and various other symptoms. Questionnaires are widely used, non-invasive, inexpensive and reliable instruments.[89,91,137–142] In IBS and other functional GI disorders, the diagnosis is based on a clinical history with typical symptoms, but diagnostic questionnaires, such as the Rome IV diagnostic questionnaire, can be used to confirm these findings, and in addition assess the frequency and severity of the symptoms. In this thesis, GI symptom severity, the level of psychological distress, central sensitization, somatization and GI-specific anxiety are all examples of symptoms rated through questionnaires by the study participants.

3.2.1 GI SYMPTOM SEVERITY

The severity of GI symptoms in IBS was assessed by two validated and widely used self-report forms in this thesis, the Gastrointestinal Symptom Rating Scale, IBS version (GSRS-IBS)[137] and the IBS Severity Scoring System (IBS-SSS).[138] These self-report instruments assess different aspects of IBS symptoms; GSRS-IBS covers a wider range of both lower and upper GI symptoms, whereas IBS-SSS is more focused on abdominal pain, and IBS-specific symptoms. The results from GSRS-IBS are normally presented as a total score, addressing the overall GI symptom severity, and five symptoms domains: abdominal pain, bloating, constipation, diarrhea, and

satiety. IBS-SSS addresses abdominal pain frequency, the severity of abdominal pain and bloating, dissatisfaction with bowel habits, and the interference on daily life caused by IBS symptoms. The results from the IBS-SSS form can be used to distinguish between mild (75-175), moderate (176-300) and severe IBS (>300). In this thesis, GSRS-IBS was used in all four manuscripts, and IBS-SSS in all manuscripts with the exception of Manuscript II.

3.2.2 PSYCHOLOGICAL DISTRESS

Psychological distress, i.e. anxiety and depression, was measured with different methods in this thesis. In Manuscripts I, II and IV, the level and presence of anxiety and depression was determined using the Hospital Anxiety and Depression scale,[89] a 14-item questionnaire, measuring the level of anxiety (HAD-A) and depression (HAD-D) on two subscales. The response options range from 0 to 3 for each question, and the total scoring of each of the subscales range from 0 (no symptoms) to 21 (maximum severity of symptoms). The scale has validated thresholds for borderline anxiety and depression (≥8), used in Manuscripts I, II and IV, as well as clinically relevant anxiety and depression (≥11), utilized in Manuscript I.

In Manuscript III, the presence and level of anxiety and depression was assessed with two other questionnaires, the Generalized Anxiety Disorder questionnaire 7-item (GAD-7) scale[143] and the Patient Health Questionnaire-9 (PHQ-9),[144] a questionnaire evaluating depression. The scoring of both GAD-7 and PHQ-9 ranges from 0 to 3 for each question, a higher score meaning more severe anxiety or depression. Both scales have a validated threshold for clinically relevant anxiety or depression (≥10).

3.2.3 SOMATIZATION

In Manuscripts I, III and IV, the level of somatization, or non-GI somatic symptoms, were measured by the Patient Health Questionnaire-12.[91] The questionnaire originates from the slightly longer version, PHQ-15,[145] but with the three GI questions removed, to exclude the influence of GI symptoms when the focus of the study is patients with GI disorders. The scoring ranges from 0 to 2 for each question, and a higher score means a higher severity of somatization /non-GI somatic symptoms. The validated threshold for patients with clinically relevant non-GI symptoms, or plausible somatization (>6), was used in this thesis.

3.2.4 CENTRAL SENSITIZATION

The presence and level of central sensitization was assessed with two questionnaires in Manuscript IV, the Central Sensitization Inventory (CSI) and the Highly Sensitive Person scale (HSP).

CSI is focused on pain sensitivity and key symptoms associated with central sensitization syndromes.[141] The CSI consists of 25 questions ranging from "Never" (0) to "Always" (4), with a higher score meaning a higher level of central sensitization. The validated threshold of ≥40[141] was used to identify participants with central sensitization.

HSP addresses sensory processing sensitivity, another facet of central sensitization, such as hypersensitivity for bright lights, noise, chemicals and stress.[82] HSP consists of 27 questions with answering options ranging from "Not at all" to "Extremely". Examples of questions are "Are you bothered by intense stimuli, like loud noises or chaotic scenes?" or "Are you easily overwhelmed by things like bright lights, strong smells, coarse fabrics, or sirens close by?". A high score equals a higher sensitivity for sensory input, and thus a higher level of central sensitization. Participants with and without central sensitization measured by HSP was distinguished by the cut-off between medium-sensitive and high-sensitive persons according to Lionetti et al. (>4.66).[140]

3.2.5 GI-SPECIFIC ANXIETY

The Visceral Sensitivity Index,[90] was used in Manuscript III as a measure of GI-specific anxiety. This special kind of anxiety originates from a fear of GI symptoms, related to the unpredictable symptom pattern commonly found in IBS. The Visceral Sensitivity Index consists of 15 items, with question scores ranging from 1 to 6. A higher score equals a more severe GI-specific anxiety.

3.2.6 OTHER QUESTIONNAIRES

In addition to the questionnaires describing GI symptoms and psychological distress, other symptoms rated with self-report forms were also utilized in this thesis. Fatigue, measured by the Multidimensional Fatigue Inventory,[146] was used in Manuscripts I and III to assess the severity of general, physical and mental fatigue, as well as reduced activity and motivation caused by fatigue. Quality of life was measured by the IBS Quality of Life questionnaire[147] in Manuscript I. Work productivity was assessed with the Work Productivity and Activity Impairment questionnaire: IBS version[148] in Manuscript I. In Manuscript III several questionnaires focusing on various psychological

factors were included. The level of perceived stress was addressed with the Perceived Stress Scale-14,[149] trait anxiety with the State Trait Anxiety Inventory, trait version,[150] pain catastrophizing with the Pain Catastrophizing Scale,[151] the presence of post-traumatic stress with relevant questions from the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders,[152] and the strengths of personality traits with the Big Five inventory.[153]

3.3 PHYSIOLOGICAL MEASURES

Physiological factors were analyzed in Manuscripts I and II, including colonic and rectal sensitivity, small intestinal motility and secretion, function of the ANS, and colonic transit. All of these measures have previously been proposed to be of importance in the pathophysiology of IBS.[34,42,43,47,48,65]

3.3.1 VISCERAL SENSITIVITY, COMPLIANCE AND RECTAL TONE

Visceral sensitivity was measured in Manuscripts I and II by two different techniques. Rectal balloon distensions were utilized for the assessment of rectal sensitivity using an electronic barostat with two different pre-programmed protocols, and colonic, or more general visceral sensitivity was measured with a combined nutrient and lactulose challenge test. In the combined nutrient and lactulose challenge test bacterial fermentation of lactulose and the effect of the Nutridrink cause gut distension and influence sensitivity through presumably different mechanisms. In both rectal distension protocols, the thresholds for first sensation and pain were used as measures of visceral sensitivity (**Figure 6**).

In the first rectal distension protocol,[118,119] isobaric phasic distensions lasting 30 seconds, with stepwise increments of 5 mm Hg were completed until the participant reported pain or until a pressure of 70 mm Hg was reached. Between each distension, a resting period of 30 seconds at the operating pressure was allowed. The operating pressure was defined as the minimal distending pressure + 2 mm Hg. Thresholds for rectal fullness, i.e. first sensation threshold, the urge to defecate, discomfort threshold and pain threshold were determined during the distensions. This protocol was used in Manuscripts I and II.

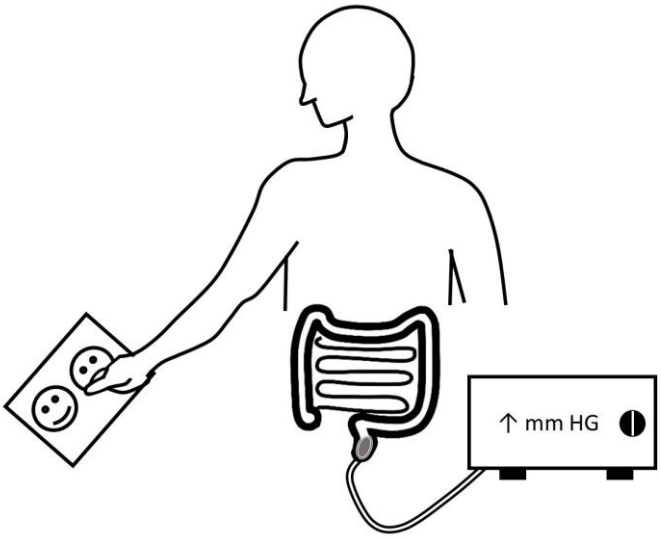

Figure 6. Rectal balloon distensions.

For the second rectal distension protocol,[118,122] a ramp inflation sequence was completed, consisting of one minute long inflations with 4 mm Hg increase in pressure between steps, until the participant described pain or until 60 mm Hg pressure was reached. The same thresholds as in the first protocol, i.e. first sensation threshold, the urge to defecate, discomfort threshold and pain threshold, were determined during this test. This protocol was used in Manuscript I.

Rectal static and dynamic compliance were assessed in Manuscript II, through calculations on the first five distension steps of the pressure-volume curve in the fasting state. The static compliance was calculated as the mean of the volume/pressure relationship, and the dynamic compliance was measured as the change in volume per change in pressure, as described by Floyd et al..[154] After the rectal distension sequence described in the first protocol, the protocol continued with an intake of a high-caloric high-fat standardized liquid meal[155] with the rectal balloon at operating pressure.[122] After the meal, the early and late rectal tone responses (0–25 minutes vs. 25–50 minutes after the meal intake) were calculated at the operating pressure, using the mean change of rectal volume for every five minutes interval after the meal in comparison with the rectal volume in the fasting state.

The combined nutrient and lactulose challenge test[156] was used for assessing visceral sensitivity in Manuscript I. At baseline and every 15 minutes during four hours after a liquid meal consisting of 400 ml Nutridrink and 25 g lactulose, the severity of abdominal pain, and digestive comfort were measured, and the area under the curve (AUC) was computed for the whole time period. These two measures were chosen due to their discriminative ability in previous studies.[157,158]

3.3.2 AUTONOMIC NERVOUS SYSTEM FUNCTION

The function of the autonomic nervous system (ANS) is only measurable through indirect measures. In Manuscript I, the heart rate variability (HRV), and in Manuscript II, the function of the baroreceptors were utilized as proxy measures of ANS function. The HRV, which is the measurable change in the time lapse between heart beats (RR intervals),[159] has previously been used as a proxy measure for ANS function in IBS.[65,114,160] The carotid baroreceptors are mechanoreceptors, reacting to changes in blood pressure, and are informing the ANS of beat-to beat changes in blood pressure.[161] The measurement of their function as a proxy measure for overall ANS function has also previously been included in IBS research.[30,116,162,163]

HRV (Manuscript I) was measured during controlled breathing to minimize the influence of the breathing pattern. First, the HRV was measured in supine position, and thereafter, the patients were requested to quickly stand up to trigger an orthostatic stress reaction, leading to increased ANS activity. After the controlled breathing protocol, 24 hours ambulatory recording without any intervention followed. The ANS balance measure, a measure of sympathetic and parasympathetic neural modulation, was extracted during the first 5 minutes after standing up. Measures of the overall HRV function during 24 hours, calculated as the standard deviation of normal-to-normal RR intervals, as well as nighttime and daytime measurements, reflecting the ANS function during rest and activity, were also extracted for analysis purposes in Manuscript I.

In Manuscript II, the baroreceptor function was analyzed through simultaneous recording of ECG, arterial blood pressure and heart rate. The baroreceptor sensitivity and baroreceptor effectiveness index were extracted. After a 10-minute long habituation period, the baroreceptor function was calculated during controlled respiration at a rate of 12 inhalations per minute to diminish the effect of spontaneous breathing on pulse and blood pressure. The baroreceptor sensitivity was calculated as the change in the intervals between heart beats in the low-frequency band (0.004–0.15 Hz) when the blood

pressure changed 1 mm Hg.[164] The baroreceptor effectiveness index was calculated as the ratio of the baroreceptor sensitivity sequences, i.e. sequences where the interbeat interval and blood pressure simultaneously increased or decreased,[164] through the total number of blood pressure increase or decrease ≥1 mm Hg during a time period.[165]

3.3.3 COLONIC TRANSIT

Colonic transit time was used in Manuscripts I and II as a measure of colonic motility. This measure was assessed through counting the number of remaining radio-opaque markers (Transit-Pellets®; Medifactia AB) in the colon following a standardized protocol. For these studies, ten ring-formed radio-opaque markers were ingested daily at 08.00 for five consecutive days. The sixth day, at 08.00 and at 20.00, five rod-formed radio-opaque markers were ingested. The following morning at 08.00, the radio-opaque markers remaining in the colon were calculated during fluoroscopy (Arcadis Avantic VC 10 A; Siemens). The number of markers were divided by the number of daily ingested markers to obtain the colonic transit time in days.[42]

3.3.4 SMALL INTESTINAL MOTILITY AND SECRETION

The motility and secretion of the small intestine was assessed in Manuscript II.[48] The small intestinal motility and secretion were calculated through antroduodenojejunal manometry with flowing electrodes in the proximal duodenum and jejunum (**Figure 7**). A reference electrode consisting of a saline filled plastic cannula was inserted subcutaneously in the left forearm. The motility and secretion were recorded for three hours in the interdigestive state and continued one hour after ingestion of a standardized meal.

The fasted and fed contraction frequencies of the small intestine were computed from the motility recordings. The mean periodicity of the fasted contractions were determined in the time period 17 minutes before the first phase III to 32 minutes after the end of phase III.[48] The fed contraction frequencies were measured during the first hour after the meal. The total time of phase III time in the interdigestive state was also calculated. The secretion of the small intestine was computed through the assessment of the potential difference, measuring mainly the flow of chloride ions, between the gut lumen and the bloodstream. The mean and maximum potential difference were assessed during late phase II to the beginning of phase III, and the rate of rise of the potential difference was measured in the first part of phase III.

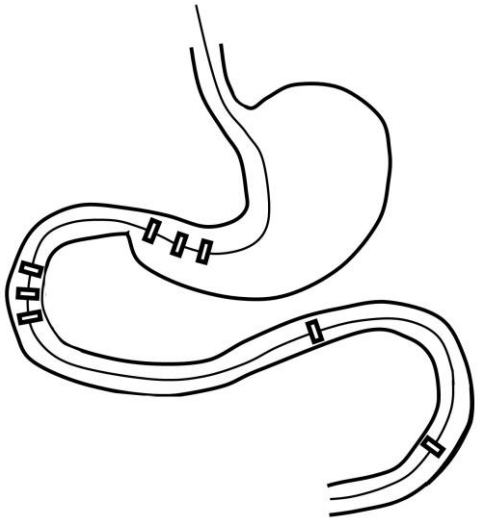

Figure 7. Schematic figure of the small intestinal motility and secretion recording catheter.

3.4 DATA ANALYSES

3.4.1 CORRELATIONS AND LINEAR TRENDS

To explore possible associations between variables in the manuscripts of this thesis, correlation analysis and one-way between-groups analysis of variance with linear contrast analysis (linear trends)[166] were used.

Due to the non-normal distribution of the dependent variable (GSRS-IBS) in all manuscripts, all analyzes of correlations were made with a correlation method for non-parametric data (Spearman's ρ). This method first ranks all data points, and then makes a correlation analysis (Pearson's r) on the ranking. The strength of the correlation coefficient ranges from weak: 0.2–0.39, moderate: 0.4–0.59, strong: 0.6–0.79, to very strong: 0.8–1.0.[167]

The linear trend analysis is a way to explore possible trends between group means.[166] This method was used in Manuscript I for describing associations between severity of psychological distress and GI symptom severity, non-GI symptoms, and quality of life in IBS patients. In Manuscript III, this method described the relations between the severity of GI symptoms and increasing

number of psychological alterations, and in Manuscript IV, it defined the link between GI symptom severity and increasing severity of central sensitization. The strength of the linear trends, i.e. the effect sizes, were described as small (partial η^2>0.01), medium (partial η^2>0.06), or large (partial η^2>0.14).[168]

3.4.2 NEUROPHYSIOLOGY SCORE

In Manuscript II, a unique score was constructed from the values of 16 different neurophysiologic factors. The overall neurophysiology score was intended to express the deviation from the normally functioning gut-brain axis in each study participant. All values considered abnormal would increase the score, and every normal factor would decrease the score, so that the overall neurophysiology score would reflect the magnitude of the deviation from the normally functioning gut-brain axis, based on the data from the study cohort. All variables were standardized by the mean to z-scores, so that each factor would influence the overall neurophysiology score equally. An individual processing of the measures followed. In some of the factors, only values below the group mean was considered abnormal and thus increased the score, i.e. a lower-than-normal rectal pain threshold, whereas the overall neurophysiology score was lowered by a high rectal pain threshold. Other factors increased the overall neurophysiology score with both values above and below the group mean, i.e. the values of rectal dynamic compliance, where both a reduced compliance and an increased compliance are viewed as abnormal. Further, some factors only increased the score with values above the group mean, whereas a value below the group mean decreased the score, i.e. psychological distress, where only a high level of psychological distress is abnormal. After individual processing of all factors, all the processed values from the neurophysiological factors were added in each patient.

3.4.3 LASSO-DERIVED SCORES

After the creation of the overall neurophysiology score described above, a computerized method was used for the elimination of insignificant factors for each of the dependent variables, i.e. the domains of GSRS-IBS. The computerized elimination method, called the Least Absolute Selection and Shrinkage Operator regression method (Lasso), extracts the factors with the strongest association with the dependent variables. The Lasso scores were used in further analyses, where the strength of the associations between the severity of GI symptoms and the Lasso scores, as well as the overall neurophysiology score described above, were compared.

The Lasso method was used to obtain a less complex model through automatic variable selection, based on shrinkage of the slope of the regression lines of simple regressions, i.e. the regression coefficients. The reduction of the regression coefficients leads to automatic variable selection through shrinkage of the coefficient estimates toward zero. The closest fit of the model, with the lowest root mean squared error, was calculated on the training set, i.e. 70% of the total cohort of Manuscript II, through a 1000 times bootstrapping procedure and 10-fold cross-validation on the test set, i.e. the remaining 30% of the total cohort. The measure of fit of the model was obtained through the root mean squared error value.

3.4.4 PSYCHOLOGICAL ALTERATIONS SCORE

In Manuscript III, the number of psychological alterations were calculated in each patient, and were added to a score describing the psychological load of the study participant. Values above validated cut-off levels or values in the uppermost tertile of the patient cohort was regarded as abnormal. The latter was based on findings in previous studies, where approximately a third of the study cohorts had abnormal values on different psychological variables.[96,109,169] In this manuscript, 18 different psychological measures, based on ten different psychological questionnaires, were analyzed. The measures with significant associations in the expected direction with at least one IBS symptom (i.e. more severe IBS symptoms in patients with versus without the psychological alteration) in the lower GI domains of GSRS-IBS (abdominal pain, bloating, constipation and diarrhea) were kept in the model. Thereafter, a false discovery rate correction for multiple comparisons was performed, and the possible collinearity (Spearman's $\rho>0.7$) of the remaining significant measures were analyzed. The number of the remaining, significant, non-collinear variables were used as a score for the psychological load in each study participant. A linear trend analysis was made between the severity of GI symptoms and the number of psychological alterations, to test the hypothesis of a cumulative effect of psychological alterations on IBS symptom severity.

3.4.5 VALIDATION ANALYSES

In Manuscript IV, a questionnaire on central sensitization, the CSI, was translated to Swedish and validated. The validation process consisted of three steps; a test-retest reliability analysis, an analysis of the construct validity and the calculation of the intraclass correlation coefficient and Cronbach's alpha.

The test-retest reliability analysis was performed in a sample consisting of IBS patients and volunteers that had completed the CSI twice, one to two weeks apart. The correlations between the individual items, and the total score of the scale between the two time points were calculated. The test-retest reliability was strong if a strong intra-person correlation occurred, or low if a weak correlation appeared. After the test-retest analysis, the construct validity of the questionnaire was calculated through a correlation analysis with a preexisting form addressing a fairly similar construct, the PHQ-15,[145] a questionnaire where many of the questions resembled questions in the CSI scale. The strength of the construct validity was rated as high ($\rho \geq 0.60$), substantial ($0.45 \leq \rho < 0.60$), moderate: ($0.32 \leq \rho < 0.45$) or low: ($\rho < 0.32$).[170] Lastly, the values for intraclass correlation coefficient and Cronbach's alpha were calculated through correlation analysis between two time points on the individual questions, and the total score of CSI, respectively. The intraclass correlation coefficient is the consistency between time points in each individual question in each study participant, whereas Cronbach's alpha was measured as the consistency between the averaged measures in each study participant.

3.4.6 MULTIVARIATE ANALYSES

In Manuscript I, a Principal Component Analysis was used as a method for visualizing differences in multivariate means between groups. In principal component analyses, no labelled dependent variable exists, as the method is an unsupervised analysis method. The grouping variable colors the score scatter for visualizing purposes but is not affecting the analysis. The goodness of the fit of the model (R^2), describes the proportion of the total variance of the model that is explained by the individual principal component. For analysis of the significance of differences between multivariate group means, a Hotelling's T^2 analysis was executed. The test is a multivariate version of the Student's t test. To extract the variables that are the most differentiating between groups, an Orthogonal Partial Least Squares-Discriminant Analysis was performed. The variables that discriminate the most between groups are shown as the variables situated furthest away from the origin in the loadings plot. The goodness of the fit of the model is described by the R^2 value, and the predictive ability of the model is shown in the Q^2 value. An R^2 value ≥ 0.5 and a Q^2 value ≥ 0.4 are deemed as sufficient in biological models.[171]

4 RESULTS AND DISCUSSION

The results from the four manuscripts of this thesis are summarized and discussed in this combined results and discussion chapter. The results from the four manuscripts are put in context in the discussion, primarily with the other manuscripts of this thesis, but also with the existing literature of the field, and the main findings of all four manuscripts are summarized in **Figure 8**.

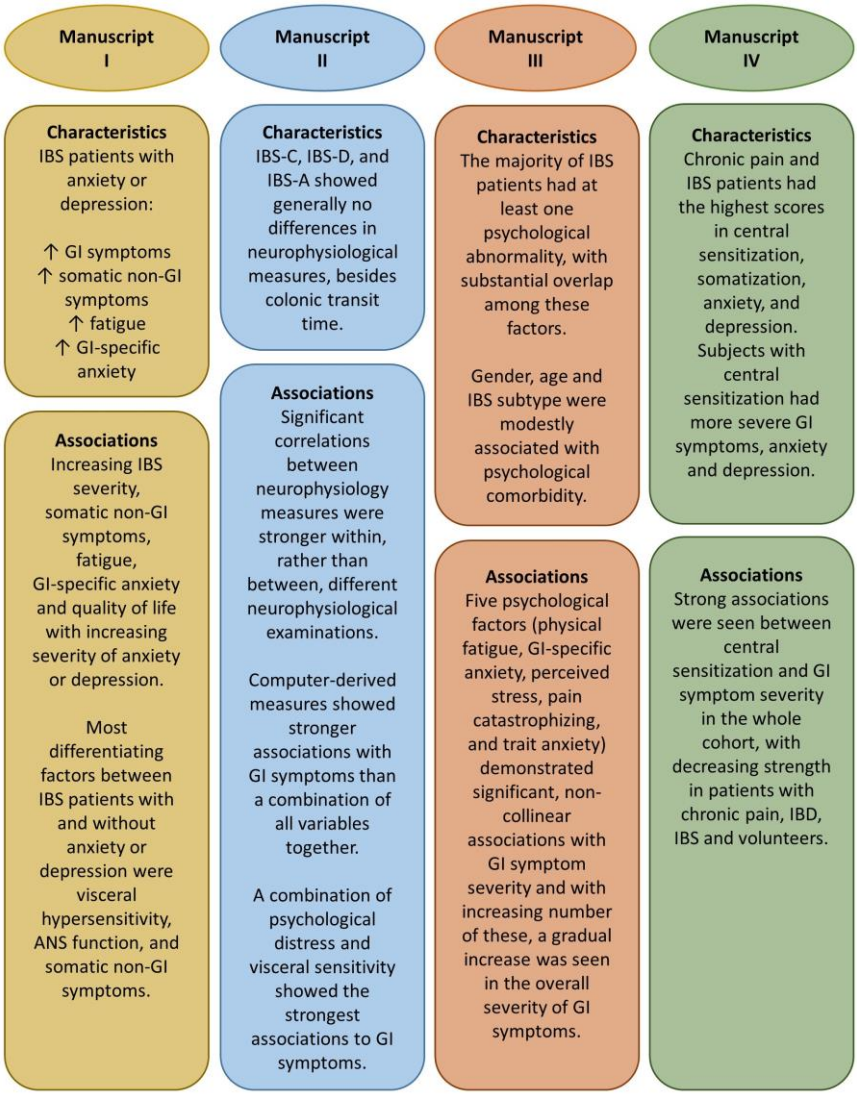

Figure 8: The main results of the four articles in the thesis.

The main findings, including characterization of the IBS patients with anxiety or depression, and the associations between GI symptom severity and other relevant measures in IBS, are the focus of this discussion and summary of results. Only the IBS patients in the four study cohorts are discussed in detail.

4.1 SUMMARY OF MAIN FINDINGS

In Manuscript I, a large cohort consisting of IBS patients with and without anxiety or depression, measured through a self-report form, was characterized. Psychologically distressed IBS patients, i.e. patients with anxiety or depression, had more severe GI and somatic non-GI symptoms, greater work impairment and reduced quality of life compared with patients without psychological distress (**Figure 9, Figure 10**). The multivariate analysis, where multiple pathophysiological measures and reported symptoms were analyzed simultaneously, revealed visceral hypersensitivity and aberrant ANS function as the most important pathophysiological differences between patients with and without psychological distress. Furthermore, IBS patients with anxiety and depression were distinguished by more severe GI-specific anxiety, higher levels of fatigue and more severe somatic non-GI symptoms when compared with patients without psychological distress.

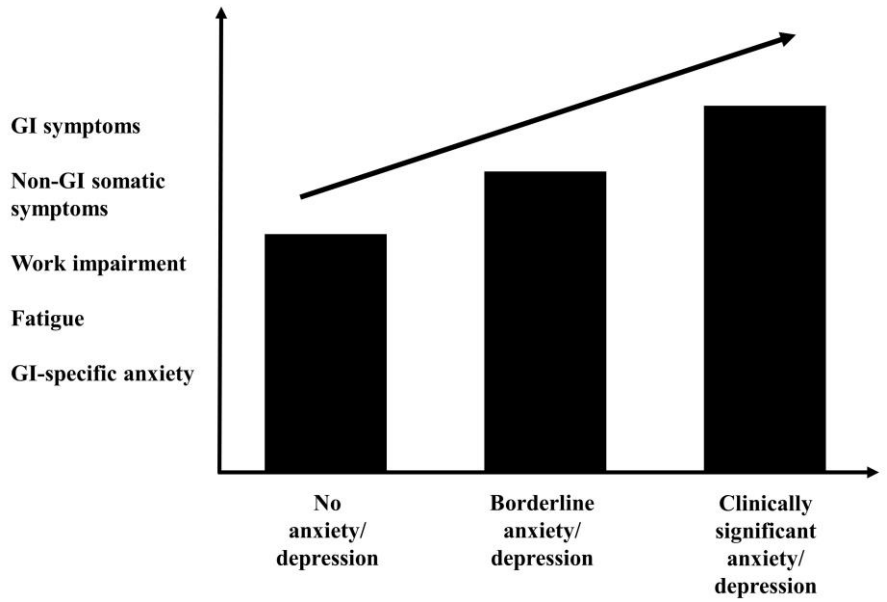

Figure 9. Summary of the results of Manuscript I, showing increasing severity of GI and non-GI symptoms, work impairment, fatigue and GI-specific anxiety in IBS patients with increasing severity of anxiety and depression.

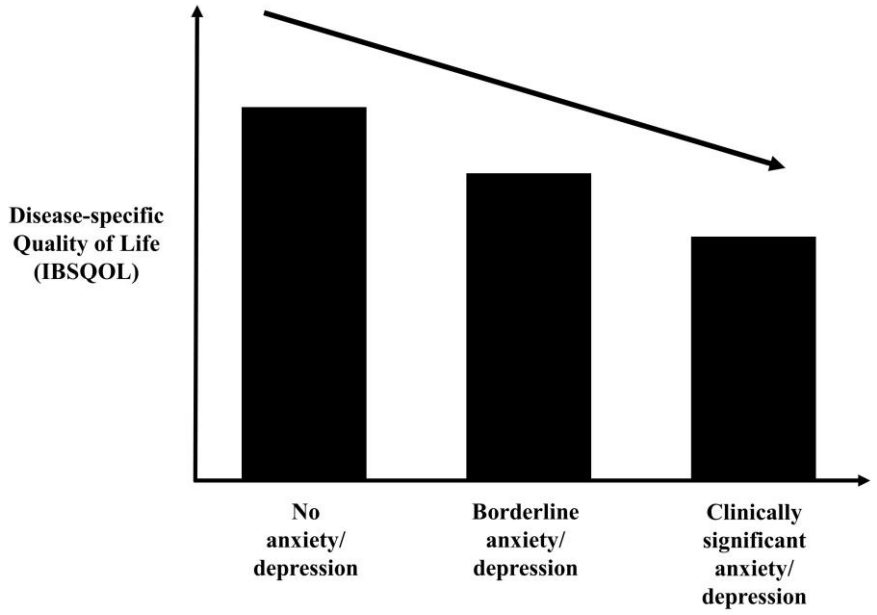

Figure 10. Summary of the results of Manuscript I, showing decreasing disease-specific quality of life (all domains) in IBS patients with increasing severity of anxiety and depression.

In Manuscript II, associations between IBS symptoms and several neurophysiological factors were explored in a cohort of IBS patients. The neurophysiology measures that were the most relevant for GI symptom severity in IBS were extracted through a computerized variable selection method. Only modest associations were seen among neurophysiology measures, and between GI symptoms and a few of the neurophysiological factors. The computer-derived combinations of the most relevant neurophysiological factors showed stronger associations than the overall score, consisting of all neurophysiology measures with GI symptom severity (**Figure 11**), but the added benefit of this variable extracting method was modest. This manuscript highlights the complexity of symptom generation in IBS, and the difficulty in understanding how physiology translates to reported symptoms.

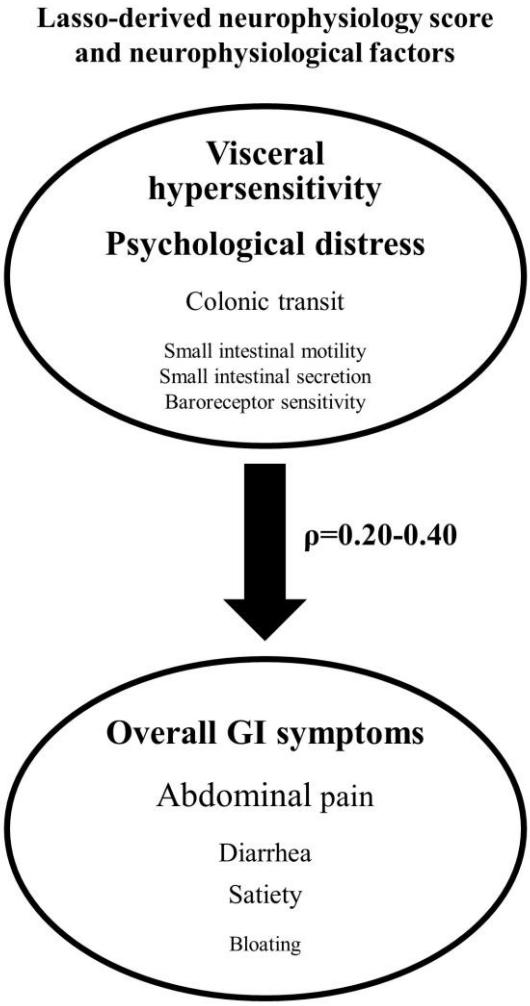

Figure 11. Summary of the results of Manuscript II, where correlations (Spearman's ρ) between variable reduced neurophysiology scores (Lasso scores) and GI symptom severity were elucidated in IBS. Neurophysiological factors that contributed most and GI symptoms that were more strongly associated with the Lasso scores are shown in larger fonts.

In Manuscript III, the associations between 18 different psychological alterations and GI symptom severity in IBS were explored. Psychological abnormalities were common in this IBS cohort, with large overlaps among these factors. The psychological measures with significant non-collinear associations surviving multiple comparisons correction were physical fatigue, GI-specific anxiety, trait anxiety, perceived stress and pain catastrophizing.

Associations with large effect sizes were seen between increasing number of these psychological alterations and overall GI symptom severity (**Figure 12**), which is in line with the current view on IBS as a multifactorial disorder of gut-brain interactions.

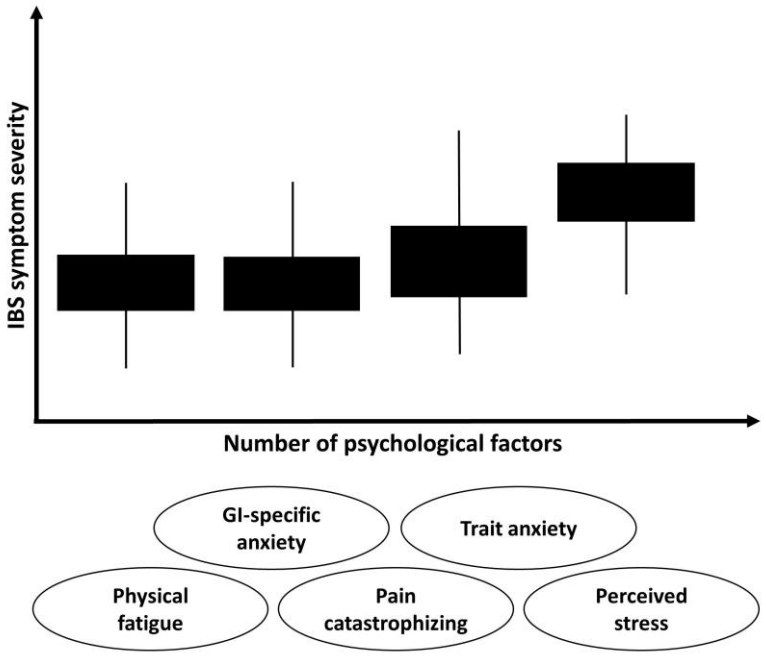

Figure 12. Summary of results of Manuscript III where a gradual increase in IBS symptom severity (measured both with IBS-SSS and GSRS-IBS) was seen with increasing number of psychological factors.

In Manuscript IV, the impact of central sensitization on GI symptoms were explored in IBS patients, and compared with patients with chronic pain and IBD, as well as volunteers without documented or self-reported chronic diseases. The presence of central sensitization was assessed in all groups, and this was found to be most common in the chronic pain group, followed by IBS patients (**Figure 13**). Study participants with central sensitization had more severe GI symptoms than participants without central sensitization (**Figure 14**). Surprisingly, the strongest associations between the level of central sensitization and GI symptom severity were not seen in the IBS group, but in the chronic pain and IBD groups, which might indicate that the presence, rather than the severity, of central sensitization is important for symptoms in IBS.

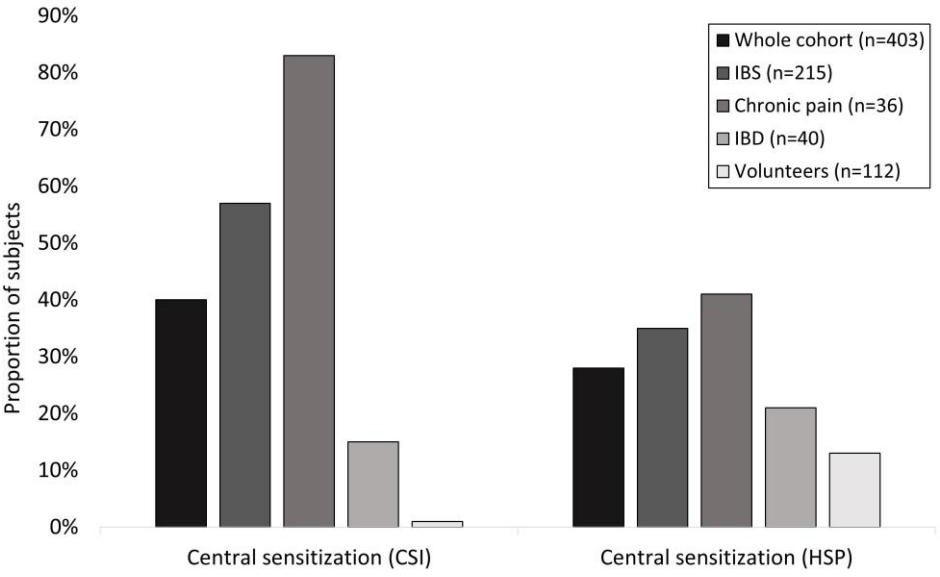

Figure 13. Summary of results of Manuscript IV showing the proportion of study participants with central sensitization according to validated cut-off levels in the CSI and HSP scales.

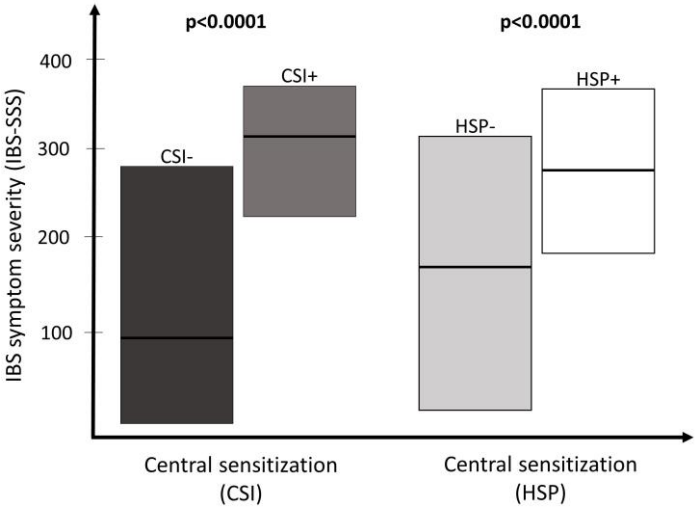

Figure 14. Summary of results of Manuscript IV showing the severity (median and interquartile range) of GI symptoms in study participants with (CSI+, HSP+) vs. without (CSI-, HSP-) central sensitization according to validated cut-off levels in the CSI and HSP scales.

4.2 SIMILARITIES AND DIFFERENCES BETWEEN THE IBS COHORTS

The severity of GI symptoms, anxiety and depression of the IBS cohorts in the four manuscripts are summarized in **Table 5**. In all cohorts, the median level of anxiety and depression were within the normal range, below validated cut-off levels for defining anxiety or depression, respectively.[143,144,172]

Table 5: Medians and interquartile ranges of GI and non-GI symptoms in the IBS cohorts of this thesis.

Measure		Manuscript I	Manuscript II	Manuscript III	Manuscript IV (IBS)	P value
Rome criteria		III	II	IV	IV	
GI Symptom severity	GSRS-IBS	3.6 [2.9-4.2]	3.5 [2.8–4.2]	4.0 [3.5-4.4]	3.8 [3.4-4.4]	<0.001
	IBS-SSS	305 [239-365]	-	312 [214-373]	317 [262-372]	0.25
Anxiety	HAD-A	7 [4-10]	6 [4–10]	-	7 [5-10]	0.20
	GAD-7	-	-	6 [3-9]	-	
Anxiety (%)	HAD-A/ GAD-7	345 (45%)	106 (40%)	56%	97 (45%)	
Depression	HAD-D	5 [2-8]	4 [2–7]	-	4 [2-6]	0.10
	PHQ-9	-	-	8 [4-12]	-	
Depression (%)	HAD-D/ PHQ-9	198 (26%)	62 (23%)	39%	41 (19%)	

Comparisons between IBS cohorts were made by Kruskal-Wallis tests. Prevalence of anxiety and depression were measured with validated cut-offs in HAD-A/ GAD-7 or HAD-D/PHQ-9.

The median severity of GI symptoms in IBS patients in the four manuscripts were in the range of moderate (GSRS-IBS) to severe (IBS-SSS) symptoms, and the variability of the severity of GI symptoms (GSRS-IBS) of the IBS patients in the four manuscripts were showing slightly different profiles (**Figure 15**). Although the median values of the GI symptom severity (GSRS-IBS) in all cohorts were not very different (**Table 5**), the overall GI symptom severity (GSRS-IBS) of the Rome IV cohorts (Manuscripts III

and IV) were slightly higher than in the Rome II and Rome III cohorts, which is in agreement with recent findings comparing GI symptom severity in Rome IV positive patients in comparison with patients diagnosed with other Rome criteria.[3,173]

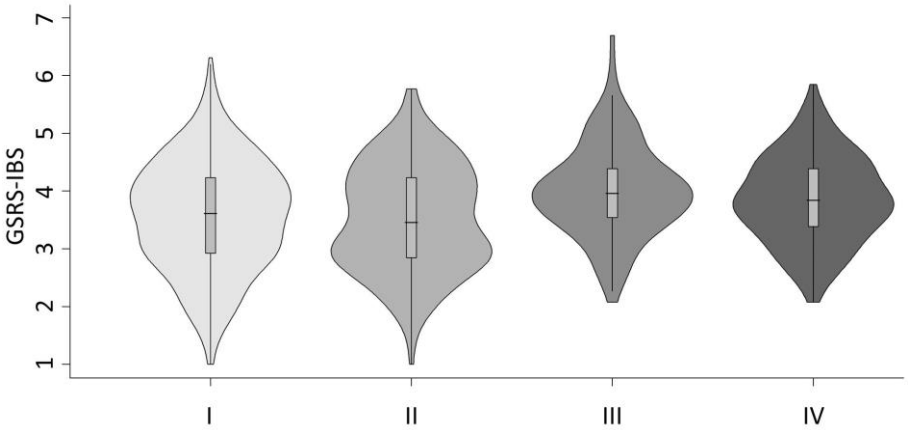

Figure 15: GI symptom severity of the IBS cohorts of this thesis measured by the Gastrointestinal Symptom Rating Scale, IBS version (GSRS-IBS). Medians and interquartile ranges shown in the boxes and the number of participants in the width of the plots.

4.3 ANXIETY/DEPRESSION

IBS patients are often afflicted by psychological and somatic non-GI comorbidities in addition to their GI symptoms.[22] One of the most studied comorbidities in IBS is psychological distress, consisting of anxiety and depression.[22,40,68] In Manuscript I in this thesis, IBS patients with anxiety and depression were characterized, and the most distinguishing measures between IBS patients with and without anxiety and depression were analyzed. In Manuscripts II, III and IV, anxiety and depression levels were assessed, and in Manuscripts II and III, psychological distress was a part of the analysis models extracting the most important factors for GI symptom severity in IBS. In all manuscripts, the median level of self-reported psychological distress symptoms was within the normal range, although the presence of anxiety or depression was high among the patients. Around half of the patients had self-reported anxiety and around a quarter had self-reported depression (**Table 5**), in concordance with prior studies.[41,68,95] No differences in the prevalence of psychological distress (HAD) were seen between the different Rome criteria cohorts, in agreement with a prior study,[3] but in disagreement with some other studies.[173,174] In Manuscript I, the focus was on analyzing and discussing the

role of psychological distress for IBS in general. In this manuscript, a range of significant measures for IBS was simultaneously analyzed in multivariate analysis, with the aim to extract the most important measures distinguishing between IBS patients with and without psychological distress. The main findings from these analyses were that patients with IBS and coexisting anxiety or depression were characterized by more severe GI symptoms, non-GI symptoms, GI-specific anxiety, visceral sensitivity, and altered ANS function. Previous studies, analyzing one or a few of the factors simultaneously, also found these measures to be of importance in IBS patients with concurrent anxiety and depression.[69,95,99,101,109,157,175] The findings from Manuscript I are also in line with findings in the other manuscripts in this thesis, where associations between psychological distress and GI symptom severity, and visceral sensitivity were seen. Furthermore, more severe anxiety and depression were detected in IBS patients with central sensitization compared with patients without central sensitization. Taken together, anxiety and depression are important factors in IBS patients, as their presence have multiple associations to GI symptom severity, non-GI symptoms and neurophysiological alterations in IBS.

In this thesis, different questionnaires were used to calculate levels of anxiety and depression, respectively. The subscales of HAD, i.e. HAD-A for anxiety and HAD-D for depression, were used for measuring anxiety and depression in Manuscripts I, II and IV, and GAD-7 and PHQ-9 assessed anxiety and depression in Manuscript III, respectively. Although results from HAD, GAD-7 and PHQ-9 are not fully comparable, an interesting detail can be noted in the higher prevalence of anxiety and depression measured by GAD-7/ PHQ-9 in Manuscript III compared with the prevalence of psychological distress measured by HAD in Manuscripts I, III and IV (**Table 5**). It has previously been argued that HAD-D and PHQ-9 may not entirely identify the same cases with depression, since only a moderate agreement are seen between their thresholds (HAD-D≥8 and PHQ-9 ≥10).[176] This might partly explain the discrepancies of depression rates between the manuscripts of this thesis, since other characteristics among the cohorts were mainly similar.

4.4 PHYSIOLOGICAL ALTERATIONS

Physiological factors previously found to have associations to IBS were assessed with multivariate analyses in Manuscripts I and II. In Manuscript I, colonic transit time, visceral hypersensitivity and ANS function were assessed in IBS patients with and without anxiety and depression. No difference between patients with and without psychological distress was seen regarding

colonic transit, although slower transit may have been expected in IBS patients with depression and faster in IBS patients with anxiety, according to a prior finding in patients with anxiety or depression.[177] The discrepancy might be caused by the small sample size and the absence of functional GI disorders in the patients of the previous study. Abnormal colonic transit has been seen in subsets of IBS patients, particularly in patients with IBS-C and IBS-D.[34,42] Furthermore, in Manuscript I, it was more common for the IBS patients with anxiety and depression to have visceral hypersensitivity, than patients without psychological distress, which is in line with previous studies.[37,157] Some differences in ANS measures were seen in patients with psychological distress in this study compared with patients without anxiety or depression. This is in contrast to a prior multivariate analysis with focus on ANS function, where the presence or absence of anxiety or depression was not associated with ANS function in IBS.[114] The discrepancy might be due to the higher number of ANS measures and more detailed ANS assessment used in the prior study.

Modest associations within measures of neurophysiological examinations and between neurophysiological parameters were found in IBS in Manuscript II. The strongest correlations were seen within examinations of rectal tone, small intestinal secretion, and visceral sensitivity (all $\rho>0.50$). ANS function and small intestinal motility showed the strongest correlation between different neurophysiology measures ($\rho=0.31$). Reported psychological distress, a proxy for CNS alterations, was associated to measures of ANS function and small intestinal motility. Colonic transit time had a significant but weak association to ANS function and small intestinal motility and secretion. Visceral sensitivity was associated with small intestinal motility. No significant connections were seen between small intestinal secretion and motility, or between ANS measures and rectal tone response. The associations between many of the neurophysiology measures have not previously been analyzed simultaneously. The findings of significant but modest associations among neurophysiological factors, and between neurophysiological factors and GI symptoms, in Manuscript II, further stresses the complexity of the symptom pattern in IBS, and highlights the lack of detailed understanding of how physiological measures translates into symptoms in IBS.

4.5 GI SYMPTOM SEVERITY

The associations between GI symptom severity and other measures were analyzed in all manuscripts of this thesis (**Table 6**). Significant relations were seen with anxiety and depression (Manuscripts I and II), psychological distress in combination with visceral sensitivity (Manuscript II), the number of

psychological abnormalities, including GI-specific anxiety, perceived stress, physical fatigue, pain catastrophizing and trait anxiety (Manuscript III) and central sensitization (CSI) (Manuscript IV). These results conclude that GI symptom severity is associated with a range of different measures in IBS, which further highlights the complexity of this multifactorial disorder.

Table 6. Associations between GI symptoms and non-GI symptoms of the cohorts in this thesis.

	I	I	II	III	IV (IBS)	IV (IBS)
	Anxiety	Depression	Psycho-logical distress + visceral sensitivity	Number of psycho-logical alterations	Central sensiti-zation (CSI)	Central sensiti-zation (HSP)
GSRS-IBS	0.076 (<0.001)	0.025 (0.001)	0.40 (<0.001)*	0.268 (<0.001)	0.24 (<0.001)*	-0.03 (0.69)*
IBS-SSS	0.044 (<0.001)	0.019 (0.002)	-	0.219 (<0.001)	0.17 (0.01)*	-0.003 (0.97)*

*Associations measured through linear trend analysis (partial η^2) or Spearman's correlation (ρ, marked with *), with p-values. Background color: orange: large effect size/strong correlation; yellow: moderate effect size/moderate correlation; green: small effect size/weak correlation; uncolored: non-significant association.*

4.5.1 GI SYMPTOM SEVERITY VS. PSYCHOLOGICAL DISTRESS

Discrepancies regarding associations between GI symptoms and psychological distress in IBS patients were seen in this thesis. In Manuscript I, significant associations were seen between these two measures when using the HAD questionnaire. In Manuscript II, psychological distress (i.e. anxiety and depression) measured with the same questionnaire was the strongest predictor of GI symptom severity together with visceral hypersensitivity. However, in Manuscript III no associations with GI symptom severity survived corrections for multiple comparisons when using the cut-off levels for the GAD-7 and PHQ-9 questionnaires to define anxiety and depression. In several previous studies significant associations have been demonstrated between GI symptoms and psychological distress.[95,100,112] However, the differences in the strength of associations seen between HAD and GAD-7 and PHQ-9 might be caused by differences in the questions included in the questionnaires and the different cut-offs proposed to define anxiety and depression, which have previously been demonstrated to not recognize entirely the same individuals with anxiety or depression.[176]

4.5.2 GI SYMPTOM SEVERITY VS. VISCERAL SENSITIVITY

Visceral hypersensitivity is common in IBS[34,119,157] and is addressed in this thesis through two different methods; balloon distension of the rectum[118] (Manuscripts I and II) and a test of colonic and more general visceral sensitivity through the combined nutrient and lactulose challenge test[156] (Manuscript I). These measures were included in the analyses based on previous studies showing association between GI symptom severity and visceral hypersensitivity, measured by rectal balloon distension[34,37,96,119] and with the nutrient and lactulose challenge tests.[156,157] Results from Manuscript II confirmed the previous findings, with significant, although modest, associations between GI symptoms and visceral sensitivity. In Manuscript I, different measures of visceral sensitivity were shown to be differently associated with anxiety compared with depression. IBS patients with anxiety showed increased visceral sensitivity through a lowered rectal pain threshold compared with IBS patients without anxiety, whereas no differences in rectal pain thresholds were seen between IBS patients with and without depression. IBS patients with depression, on the other hand, reported more abdominal pain and thus higher visceral sensitivity, than IBS patients without depression during the nutrient and lactulose challenge test, whereas no differences between patients with and without anxiety were seen. In the multivariate analyses in Manuscript I, the level of visceral sensitivity measured with the nutrient and lactulose challenge test was one of the strongest discriminating measures between patients with and without psychological distress.

4.5.3 GI SYMPTOM SEVERITY VS. PSYCHOLOGICAL ALTERATIONS

The associations between GI symptoms and altered psychological measures beyond anxiety and depression were elucidated in detail in Manuscript III. Several of the psychological measures with previously demonstrated alterations in subsets of IBS patients showed individual associations with GI symptom severity in this thesis. Pain catastrophizing, GI-specific anxiety, trait anxiety, perceived stress and physical fatigue demonstrated significant, non-collinear associations with GI symptom severity, and were cumulatively related to the severity of GI symptoms, in accordance with previous studies.[21,109,112,113,178]

4.5.4 GI SYMPTOM SEVERITY VS. CENTRAL SENSITIZATION

The presence of central sensitization was high in IBS (Manuscript IV), but the association between the level of central sensitization with GI symptom severity was modest. The interpretation of this partly surprising finding is that the presence, rather than the severity, of central sensitization may be of importance for GI symptoms in IBS. No prior studies have so far addressed associations between central sensitization, measured with questionnaires, and the GI symptom severity in IBS, and therefore, confirming studies of these results are warranted.

4.5.5 GI SYMPTOM SEVERITY VS. OTHER FACTORS

Other neurophysiology measures, earlier proposed to be of relevance in IBS, and therefore interesting to explore in terms of their associations to GI symptom severity, showed no or weak associations with GI symptoms in Manuscript II. Abnormalities in the rectal tone response and rectal compliance have previously been demonstrated in IBS,[51,123,125,179] but here, only one weak association between rectal compliance and the bloating domain of GSRS-IBS was seen. The rectal tone response and rectal compliance showed weak but significant correlations with each other, and weak associations to small intestinal sensorimotor parameters. No significant associations were seen between GI symptom severity and ANS function, or measures of small intestinal motility or secretion. In line with previous studies, modest but significant associations were seen between colonic transit and bowel habits in IBS.[42] However, in the multivariate analysis, ANS function, small intestinal secretomotor function and colonic transit time were all significant for one or more of the Lasso derived neurophysiology scores, potentially indicating that these factors may together with other physiological measures influence symptoms in IBS.

4.6 COMPARISON OF QUESTIONNAIRES MEASURING GI SYMPTOM SEVERITY

IBS-SSS and GSRS-IBS are two often used questionnaires addressing the severity of GI symptoms in IBS. Both of these were used in the majority of the manuscripts of this thesis, as they focus on different aspects of GI symptom severity. The associations between GI symptom severity and anxiety or depression (Manuscript I) and the number of psychological alterations (Manuscript III) were somewhat stronger when the overall GI symptom

severity was measured with GSRS-IBS, compared with IBS-SSS. This might be explained by differences in the construction of the questionnaires. IBS-SSS is focused on pain, as two of five questions measure pain, whereas GSRS-IBS covers a broader range of GI symptoms. Moreover, in GSRS-IBS constipation and diarrhea are addressed separately, while IBS-SSS only assesses overall dissatisfaction with bowel habits. In the IBS cohorts of this thesis, the most severe GI symptom measured by GSRS-IBS was bloating, followed by abdominal pain (Manuscripts II and III). These two domains also showed the strongest associations with central sensitization in Manuscript IV. In general, the constipation domain of GSRS-IBS had weaker or fewer associations with the measures of interest compared with the other domains, indirectly indicating that measures of relevance for constipation (e.g. anorectal function) were not included in these studies. (Manuscripts II and III).

4.7 LIMITATIONS

A limitation of this thesis is the use of proxy measures to describe the level of central sensitization, and to assess the function of ANS and CNS. CNS function and dysfunction and central sensitization can be measured with brain imaging[33,66,180,181] and conditioned pain modulation for central sensitization.[182] These methods are costly and not readily available, and therefore, self-report forms have been developed as an alternative way to assess psychological distress, which can serve as a proxy for CNS function, and central sensitization of the patient.[82,89,141,183,184] The forms are widely used, valid and reliable.[143,144,172] ANS function was also solely assessed with proxy measures. Heart rate variability and baroreceptor function have been commonly used to address the function of the ANS in IBS and other disease states,[114,163,185–188] although the possible vagueness of these measures has been discussed.[189] The addition of other factors to the measures used in this thesis could broaden and deepen the understanding of the pathophysiology of IBS. Although an extension of parameters could augment the risk of noise in the analytical models, computerized methods aiming at the reduction of factors, such as the Lasso method applied in Manuscript II, could be used to identify the relevant factors for the outcome measures.

Unfortunately, no conclusions of the causality of GI symptoms and psychological distress or other measures used in the analyses can be drawn from the studies in this thesis, as all are cross-sectional. Longitudinal studies, providing information regarding cause and effect are highly warranted, as only a few longitudinal studies in IBS populations exist. The severity of GI symptoms was found to be significantly but modestly associated with the level

of psychological distress one year later at all five yearly measurements in one study.[190] In a study with a 12 year follow-up assessing anxiety, depression, and the presence of IBS, baseline anxiety and depression were predicting IBS 12 years later, but not vice versa, indicating that the brain-gut pathway, rather than the gut-brain pathway, is the most influential pathway in IBS.[108]

A proportion of the study participants did not complete all questionnaires or did not undergo all neurophysiological and pathophysiological examination in the four manuscripts of this thesis, which is a limitation. To assess as large as possible study cohorts with complete data, computerized multivariate imputation methods were used to obtain reliable approximate values for the missing data in the study cohorts of Manuscripts I and II.[191–193]

The interpretability of the results from this thesis might be limited by the structure of GSRS-IBS, the most used outcome measure of this thesis. It is a Likert scale questionnaire with a limited number of response options. Although the seven options give a less-than-preferred level of detail in the analysis results, this form is widely used in IBS research, which facilitates the comparison of results between studies. Furthermore, the generalizability of the results from this thesis may be questioned, as all cohorts consists of moderate/severe IBS patients investigated in a secondary/tertiary care center. However, the IBS patients in these studies were mostly referred from and followed up in primary care. Therefore, these findings could be generalizable to primary as well as secondary/tertiary care patients with moderate/severe IBS, but not to subjects with mild symptoms.

5 CONCLUSION AND FUTURE PERSPECTIVES

IBS is a multifactorial disorder with a wide range of individual symptoms accompanying the disease-characterizing symptoms defining the disorder, i.e. abdominal pain and altered bowel habits. The varying extraintestinal and intestinal symptoms in IBS generates a heterogeneous and complex disease picture. A variety of these symptoms, including their potential underlying physiological alterations, and their associations with, and relevance for, the severity of IBS symptoms, have been explored in this thesis, expanding the knowledge of factors of importance for GI symptoms in IBS.

Psychological distress, i.e. anxiety and depression, was found to be common in IBS. IBS patients with anxiety and depression showed more severe GI symptoms, extraintestinal symptoms, and lower quality of life compared with patients without psychological distress. These findings highlight the importance of addressing the psychological state of the IBS patient, as well as the GI symptom severity, in the patient management.

The most prominent neurophysiology measures associated with GI symptom severity in IBS identified in this thesis were visceral hypersensitivity and psychological distress, a proxy measure for aberrant CNS function. A simultaneous analysis of the associations between GI symptom severity in IBS and these two factors, together with other factors also shown to be of potential importance in IBS, such as the microbiota composition, gut immune and permeability parameters and environmental factors, would be of interest to assess in future studies to expand the understanding of symptom generation in IBS.

Psychological alterations were found to be cumulatively associated with GI symptom severity in IBS patients. Future studies elucidating the association between GI symptoms in IBS and detailed aspects of psychological alterations would be of interest. For example, for somatization, the relevance of somatic pain situated in different bodily locations could be better characterized in IBS, and for GI-specific anxiety, the level of avoidance of trigger foods in IBS and the relevance of food avoidance for symptoms, could be of particular interest, since food-related symptoms are central for many IBS patients.[24] Furthermore, simultaneously analyzing multiple psychological alterations and neuro-physiology measures could further elucidate the most important factors for GI symptom severity in IBS.

Central sensitization was found to be common in IBS, but the level of central sensitization had only modest associations to GI symptom severity. Unfortunately, this study primarily included IBS patients with moderate and severe symptoms, which might partly explain this modest association. Future studies including IBS patients of all levels of symptom severity might give a fairer picture of the associations between GI symptoms and central sensitization and might enhance the understanding of the role of central sensitization for GI symptoms. Also warranted are studies using both questionnaires and more objective measures of central sensitization, with the aim of further revealing if the presence and absence of central sensitization, rather than the level, is important for IBS. Lastly, a more detailed exploration of the relative importance of different aspects of central sensitization, e.g. lower back pain, migraine, sensitivity for lights and sound, for GI symptoms and well-being in IBS could be of clinical benefit for IBS patients and could lead to improvement in IBS management.

Today, IBS patients are subgrouped into four categories based on the predominant bowel habit. Other types of subgrouping have been proposed because of the heterogeneity of the disorder, based on the theory that IBS is a group of diseases with similar symptoms rather than one specific disease. Subgrouping based on the severity of abdominal pain has been previously examined,[194] as has the division of patients based on bowel habit predominance, together with the severity of psychological and extraintestinal symptoms.[195–197] My suggestion, based on the findings in this thesis, would be to evaluate the subgroups of IBS patients based on the presence of anxiety, depression, visceral sensitivity, and central sensitization. These new subgroups should first be characterized, and thereafter, GI symptom pattern, work productivity, quality of life, and alterations in other pathophysiological measures should be assessed in order to explore the differences between the groups. Longitudinal studies should also be performed to define the relevance of these subgroups for health care consumption, response to therapy and prognosis of the disease.

In conclusion, in this thesis many factors that have previously been analyzed individually in IBS were simultaneously assessed in complex analyses revealing factors of importance for the severity of GI symptoms in IBS, and how they interact. Various psychological and physiological measures were demonstrated to be associated with and of potential relevance for GI symptom severity in IBS. This thesis strengthens the view of IBS as a disorder of gut-brain interactions and increases the knowledge essential for further improvement of successful individualized treatment plans in IBS patients.

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