Exploring the Biopsychosocial Model in Irritable Bowel Syndrome

with emphasis on stress, comorbidities and fatigue

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To my family

In the end only kindness matters.

Jewel

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ABSTRACT

Background

Irritable bowel syndrome (IBS) is a common, chronic, relapsing, and sometimes disabling, symptom-based disorder of gut brain interactions. It has got a female predominance and occurs in all ages, with a slight decrease among elderly. The IBS symptoms can affect everyday work and social life in addition to an increased use of health care resources. Most IBS patients are diagnosed and helped in primary health care (PHC). For many patients, available treatment is insufficient. It is known that both extraintestinal symptoms such as fatigue, as well as comorbidities such as mood disorders, chronic pain syndromes, and insomnia contribute to the illness burden, often to a larger extent than the gastrointestinal symptoms as such.

Eventhough the pathophysiology of IBS is not completely known, it is now conceptualized as a disorder of altered brain-gut interactions, where a biopsychosocial model helps in understanding the symptoms. Exposure to stress is thought to play an important role overall in the pathology of IBS, as well as immune activation at least in a subgroup of patients.

This thesis aimed to gain deeper understanding of the biopsychosocial mechanisms of IBS and its associations with stress, comorbidities, and fatigue.

Methods

Study I and II are based on the Twin cities IBS study population, which included IBS patients and a control group of other patients without gastrointestinal complaints from ten PHC centres in the county of Östergötland. Alongside demographics, psychosocial questionnaires and a GI symptom diary, it included analyses of hair cortisol concentrations (HCC) evaluated in study I, and data on self-rated health as well as diagnoses of comorbidities, and number of health care contacts from a regional registry, evaluated for study II.

Study III of this thesis is based on the Brain-Gut study with a population of secondary care IBS patients, and healthy controls (HC). It included self-rated measures of fatigue impact on the daily life and early adverse life events, as well as measures of circulating TNF- α , and analyses of resting-state functional magnetic resonance imaging of brain areas within a mesocorticolimbic circuitry of known relevance for fatigue.

Results

Study I: Perceived stress was higher in the IBS group while a considerable portion of IBS patients had low levels of HCC. No association between perceived stress and HCC was seen in either group.

Study II: IBS patients had lower self-rated health and more PHC utilization than the non-IBS patients. Good self-rated health was independently associated with younger age, higher sense of coherence and less gastrointestinal pain in both groups. In IBS, PHC utilization was associated with comorbidities in general, and sleep disorders in particular.

Study III. Fatigue impact on daily life, and TNF- α were higher in IBS patients than in HC. In IBS, further an association was seen between fatigue impact on the one hand, and TNF- α , emotional abuse in childhood, as well as altered mesocorticolimbic connectivity on the other.

Conclusion

In conclusion this thesis firstly emphasizes that IBS patients in many ways, including health outcomes, consists a vulnerable group of PHC patients. We add evidence for a possible suppression of the stress response system in a substantial portion of IBS patients.

Further, comorbid sleep disorders seem to be particularly associated with excess PHC utilization in IBS, and could possibly be a target for treatment interventions. Moreover, alongside treating gastrointestinal pain, efforts to improve the individuals' sense of coherence could be one way to achieve better self-rated health in both IBS and non-IBS patients.

Finally, we suggest that fatigue in IBS is associated with immune activation, central alterations and to some extent also previous childhood trauma.

SVENSK SAMMANFATTNING

IBS (Irritable Bowel Syndrome) är en vanlig, kronisk tarmsjukdom med återkommande symtom av buksmärta tillsammans med ändrade avföringsvanor. Den drabbar cirka 10% av befolkningen och är vanligast hos kvinnor samt något mindre vanlig hos äldre. För många innebär IBS ett stort lidande, försämrad livskvalitet och ökad sjukvårdskonsumtion. De flesta IBS-patienter tas om hand i primärvården. Nuvarande, tillgänglig behandling är ofta otillräcklig.

En del IBS-patienter har också andra symtom, så som muskelsmärta och uttalad trötthet, som inte går över vid vila, så kallad fatigue. Det är också vanligt förekommande med andra sjukdomstillstånd såsom depression och andra smärtsyndrom. Det är visat att den typen av symtom och samsjuklighet många gånger är värre för IBS-patienten än de faktiska magtarmsymptomen.

Numera tror man att symtomen vid IBS beror på störningar i det ömsesidiga samspelet mellan tarm och hjärna, men de bakomliggande mekanismerna är inte helt klarlagda.

För att förstå IBS-patientens symtom är en så kallad biopsykosocial förklaringsmodell till stor hjälp, då symtomen inte bara beror av biologiska mekanismer, utan individens sociala miljö samt psykologiska reaktioner spelar också stor roll.

Stress är centralt för så väl utveckling av, som symtom vid IBS och även för förståelsen av den biopsykosociala förklaringsmodellen generellt. Tidigare forskning visar också att immunsystemet är påverkat hos åtminstone en andel av IBS-patienterna.

Syftet med den här avhandlingen var att nå en djupare förståelse av biopsykosociala mekanismer vid IBS med fokus på stress, samsjuklighet och fatigue.

Delstudie I och II jämförde IBS-patienter och en kontrollgrupp med andra primärvårdspatienter, utan magtarmsymtom på 10 vårdcentraler i Östergötland. Delstudie III undersökte IBS-patienter på magtarmkliniken i Linköping i jämförelse med friska kontroller.

I delstudie I undersökte vi kortisol i hår-nivåer, som ett mått på hur stresshormonnivåerna varit över tid. Trots att IBS-patienterna som grupp beskrev en större självupplevd stress än icke-IBS-patienterna hade en andel förhållandevis låga nivåer av kortisol i håret.

I delstudie II undersökte vi hur samsjuklighet i form av totalt antal registrerade diagnoser, samt vissa specifika diagnoser och psykologiska aspekter samt magtarmsymtom, påverkade självskattad hälsa och primärvårdskonsumtion hos IBS- och icke-IBS-patienterna. I båda grupperna var lägre ålder, större känsla av sammanhang, och lägre grad av buksmärta oberoende associerat med bättre självskattad hälsa. Hos IBS-patienterna fann vi närmast en femfaldigt ökad risk att ha många kontakter med primärvården vid samtidigt diagnostiserad sömnstörning. Den kopplingen sågs inte hos patienterna utan IBS. Däremot var det totala antalet diagnoser också en faktor av betydelse för vårdkonsumtionen i båda grupperna.

I delstudie III belyste vi fatigue och eventuella kopplingar till nivåer av en proinflammatorisk signalmolekyl (TNF- α) samt till självrapporterade missförhållanden under uppväxten hos IBS patienter och friska kontroller. Med funktionell magnetkameraundersökning av hjärnan undersökte vi också kopplingen mellan aktivitetsmönster i områden som är relaterade till emotionella, kognitiva och motivationsrelaterade aspekter av fatigue hos IBS patienter och friska kontroller. Vi fann att IBS-patienterna upplevde fyrfaldigt mer påverkan av fatigue på sina dagliga liv än kontrollerna. Den ökade trötthetsupplevelsen var också relaterad till högre nivåer av TNF- α i blodet hos IBS-patienterna, liksom i viss mån till missförhållanden under uppväxten. Slutligen såg vi att högre grad av upplevd fatigue hos IBS-patienterna ledde till minskad samtida aktivitet (konnektivitet) i de undersökta hjärnområdena som representerade de motivationsrelaterade och kognitiva aspekterna av trötthetsupplevelsen. Någon liknande koppling mellan TNF- α och förändringar i hjärnaktiviteten sågs ej.

Sammanfattningsvis pekar våra resultat gällande samsjuklighet, självskattad hälsa, psykosociala faktorer samt fatigue på att IBS-patienterna är en sårbar patientgrupp som bör uppmärksammas.

Våra resultat från delstudie I pekar vidare mot att stressaxeln kan vara uttröttad hos vissa IBS-patienter.

Enligt resultaten i delstudie II är känsla av sammanhang jämte buksmärta faktorer, som bör tas i beaktande för att om möjligt uppnå bättre självskattad hälsa både hos IBS- och andra primärvårdspatienter. Vidare synes IBS-patienternas förhållandevis stora vårdkonsumtion vara särskilt avhängig av samtidig sömnstörning, vilket också det torde vara av betydelse i klinisk praxis och av intresse för framtida forskning.

Delstudie III, visar ett samband mellan fatigue, som bisymtom vid IBS, och så väl barndomstrauman som möjlig immunaktivering. Vi fann även att IBS patienter som upplevde stor påverkan av fatigue på sina dagliga liv, också hade en minskad konnektivitet mellan hjärnstrukturer av betydelse för kognition och motivation.

LIST OF PAPERS

I. Cortisol levels in hair are altered in irritable bowel syndrome - A case control study in primary care

Anna-Karin Norlin, Susanna Walter, Elvar Theodorsson, Valerie Tegelström, Ewa Grodzinsky, Michael P. Jones, Åshild Faresjö

J Psychosom Res. 2017 Feb;93:69-75.

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II. Primary healthcare utilisation and self-rated health among patients with Irritable Bowel Syndrome: What are the impacts of comorbidities, gastrointestinal symptom burden, sense of coherence and stress?

Anna-Karin Norlin, Åshild Faresjö, Magnus Falk, Michael P. Jones, Susanna Walter

J Psychosom Res. 2019 Apr;119:1-7.

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III. Fatigue in irritable bowel syndrome is associated with plasma levels of TNF- α and mesocorticolimbic connectivity

Anna-Karin Norlin, Susanna Walter, Adriane Icenhour, Åsa V Keita, Sigrid Elsenbruch, Olga Bednarska, Michael P. Jones, Roza-lyn Simon, Maria Engström

Under revision for *Brain Behav Immun*

ABBREVIATIONS

ACC	anterior cingulate cortex
amINS	anterior-middle insulae
ACTH	adrenocorticotrophin
ANS	autonomic nervous system
BMI	body mass index
CRF	corticotrophin-releasing factor
CNS	central nervous system
CTQ	childhood trauma questionnaire
DLPFC	dorsolateral prefrontal cortex
fMRI	functional magnetic resonance imaging
FDR	false discovery rate
GI	gastrointestinal
HPA axis	hypothalamic-, pituitary-, adrenal axis
HCC	hair cortisol concentrations
IBS	irritable bowel syndrome
IBS-SSS	IBS severity scoring system
IL	interleukin
IFN	interferon
IQR	interquartile range
MacArthur	MacArthur scale of subjective social status
MRI	magnetic resonance imaging
NAc	nucleus accumbens
PHC	primary health care
PSS	perceived stress scale-14
PVN	paraventricular nuclei
ROI	region of interest
ROC	receiver operating characteristic
rsfMRI	resting-state fMRI
SOC	sense of coherence
TNF	tumor necrosis factor
TWIBS	twin cities IBS

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A personal reflection and background

As a general practitioner engaged in primary care, you meet all sorts of people and medical dilemmas. Some patients seem to be more severely affected with all kinds of diseases, disorders, and symptoms, sometimes leaving the doctor with a sense of inadequacy. According to my experience in those cases alongside somatic pain syndromes, functional gastrointestinal (GI) diseases, or disorders of brain-gut interaction as they are more recently known, are often present. As a young doctor I had a wish to better understand and to help this group of patients. In the year 2010, I had the privilege to join the research study Twin Cities IBS (TWIBS) through including patients with Irritable Bowel Syndrome (IBS) from my primary health care (PHC) center. Little did I know that the journey towards this PhD-thesis had started.

Since then, it has been ten intensive and educational years coming to grips with new research fields, that I actually knew quite little of beforehand. I have met many inspiring researchers, while my own PhD-path, all the time has been taking new and interesting directions, ending up in a few impasses, however still always leading to further knowledge and experience. Working with the TWIBS study lead to the first two papers of this thesis. The results, highlighting a possible altered stress-axis as well as comorbidities in general, and sleep disorders in particular, finally led the course of my PhD-path towards the extraintestinal symptom fatigue, and its association with neuro-immunological mechanisms.

Parallel to my involvement in the TWIBS-study, I had closely followed the process of the Brain-Gut study being developed at the gastrointestinal clinic. Eventually I could also take part in that study for the last part of this thesis. With that, I dove right into the interesting research field of sickness behavior following immune activation

Trying to get the full picture of the areas studied in this thesis, at the time of an ongoing pandemic, surely has added extra insight, or a sense of coherence really. It is not hard to understand that pathogens have been the biggest threats to our ancestors, and that a pattern of behavior has developed, and filled its purpose supporting the immune defense.

Finally, all through this thesis, when studying the vast bulk of previous literature regarding IBS, stress, comorbidities and fatigue, the putative underlying influence of previous childhood trauma kept appearing, which eventually made me add some additional analyses regarding associations between early adverse life events, fatigue and immune activation.

INTRODUCTION

Irritable Bowel Syndrome

IBS is a chronic, relapsing and sometimes disabling disorder of gut brain interactions, defined by presenting symptoms, and best understood with a biopsychosocial model in mind (1). It affects roughly about 10% of the general population with a female predominance and the majority of patients being diagnosed in primary care (2-4). It occurs in all ages with a slight decrease among elderly (4). Quality of life among IBS patients is in many cases seriously impaired, and IBS symptoms can affect every day work and social life in addition to an increased use of healthcare resources (5, 6). Even though not life-threatening or causing serious physical harm in itself, a previous study has estimated that, on average, patients would give up 10 to 15 years of their life expectancy for an instant cure for IBS (7).

History and diagnostic criteria of IBS

The diagnosis of IBS is based on diagnostic criteria in the absence of alarm symptoms, and after exclusion of differential diagnoses such as inflammatory bowel disease, coeliac disease, and colorectal cancer. This can be reached with a minimal of laboratory tests and, if appropriate, colonoscopy (1, 8).

Historically a disorder that sounds like IBS was mentioned as early as 1849 by Cumming: “The bowels are at one time constipated, another lax, in the same person. How the disease has two such different symptoms - I do not profess to explain”(9). In 1892 Osler named this syndrome mucous colitis and in the 1920s it was called colonic spasm. In 1929 Jordan and Kiefer introduced the term irritable colon, which later changed to colon irritable and finally to the current used terminology: Irritable bowel syndrome (10).

Over the last 40 years since the first release of the Manning criteria in 1978 (11), IBS criteria have been repeatedly and substantially altered according to worldwide epidemiological studies and derived from consensus processes by a multinational group of experts in functional gastrointestinal disorders (12-18). While this thesis has developed, also the Rome criteria have changed from Rome III, used for the studies of this thesis, to the current Rome IV. Differences are described in Box 1. Basically, the criteria are sharpened leaving out “discomfort”, considered to be imprecise. The current Rome IV criteria also allows any relation (both improvement as well as deterioration) to defecation. At the same time the general terminology is shifting from the long-used concept of “functional gastrointestinal disorders” towards, with current knowledge, more correctly referring to “disorders of gut-brain, or brain-gut, interaction”(1). For the guidance of

treatment and to improve homogeneity of patients recruited to clinical trials, IBS is further classified into four subtypes according to stool consistency (IBS with diarrhea, IBS with constipation, IBS with mixed symptoms of constipation and diarrhea or unsubtyped IBS) (1, 8).

Box 1. Rome III and IV Criteria for Irritable Bowel Syndrome	
ROME III criteria	ROME IV criteria
Recurrent abdominal pain or <u>discomfort*</u> , at <u>least 3 days/ month</u> associated with 2 or more of the following**:	Recurrent abdominal pain on average <u>at least 1 day per week</u> , associated with 2 or more of the following**:
<u>Improvement</u> with defecation	<u>Related</u> to defecation
<u>Onset</u> associated with a change in frequency of stool	Associated with a change in frequency of stool
<u>Onset</u> associated with a change in form (appearance) of stool	Associated with a change in form (appearance) of stool
<i>*Discomfort means an uncomfortable sensation not described as pain.</i>	
<i>**Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.</i>	

Description of Rome III and IV criteria. Differences are underlined. Adapted from Longstreth et al. 2006 (19), Drossman et al. 2016 (1).

IBS pathophysiology

Probably due to its heterogeneity, the pathophysiology of IBS is not yet completely clear. Disturbances of the brain-gut axis, associated with any combination of altered central nervous system processing, visceral hypersensitivity, motility disturbances, altered mucosal immune function and/or gut microbiota, have been suggested as possible contributing mechanisms behind the disease (1, 8, 20-22)

Several predisposing factors have been identified. Those include genetics, gender, environmental and psychosocial factors. There are also several known triggering factors such as gastroenteritis, trauma, stress and food intolerances (22, 23). Of note is that IBS is a heterogeneous disorder and identical symptoms in different patients can be due to different disease processes (22).

Central to the pathophysiology of IBS is the bidirectional interactions between the central nervous system (CNS) and the enteric nervous system of the gut. Most recent knowledge also focuses on reciprocal interactions

between brain networks and networks of multiple cells in the gut, including microbiota (20, 24). In this, the gut-to-brain communication is thought to be mediated by neural, endocrine and inflammatory pathways, while the brain primarily relies on the autonomic nervous system in communicating with the gut (20, 21). Signals from the gut to CNS are of importance for optimal digestion and reflex activation of the GI tract and is of importance for general well-being of the individual. Through brain imaging studies, different networks of direct importance for processing of the visceral signals have been detected (21). Those are primarily located in the frontal cortex and the insula. In this, it has been suggested that IBS patients lack the ability to down-regulate real and anticipated reactions of visceral pain resulting in a reinforced pain experience (23, 25).

Treatment of IBS

There is no cure nor ultimate treatment for IBS. A person-centered, step-wise management approach is recommended (26). General management principles can be sufficient for patients with mild symptoms. That includes making a confident diagnosis alongside offering explanation and reassurance as well as lifestyle and dietary advice. With lasting moderate-to-severe symptoms further pharmacotherapy on the basis of symptoms is the next step. Finally, in the most severe cases with symptoms refractory to the above measures and to those with comorbid extraintestinal symptoms, psychotropic drugs and psychological treatment alternatives can be effective. A helpful basis for effective treatment is a good physician-patient relationship, sometimes known as a therapeutic alliance (1, 8, 26).

The biopsychosocial model of IBS and the specific aspects investigated in this thesis

The art of medicine is not limited to knowledge about biological functions and their dysregulation. In all illness, disease and the perception thereof, alongside biological mechanisms are also psychological and sociological components of importance for both understanding the underlying pathophysiology and for determining proper treatment (27-29). Due to the lack of a framework for those dimensions of medicine, in the late 1970's George Engel published a landmark paper in *Science* proposing a biopsychosocial model of illness and disease to use in research, teaching and the real world of health care (27, 29). Since then this model is widely used and this way of understanding illness and disease is particularly applicable to IBS, as well as other pain syndromes, in psychiatry, and in rehabilitation (30-32).

IBS is now conceptualized as a disorder of altered brain-gut interactions (1), where a biopsychosocial model helps in understanding the

symptoms (30, 33, 34). Even though evidence for possible underlying organic alterations in IBS are emerging (22, 35), all three dimensions of the biopsychosocial model are still important to keep in mind. Figure 1 illustrates how biological, psychological, and social factors interact to cause GI as well as extraintestinal symptoms and subsequent health outcomes in IBS patients. Factors that are particularly investigated in this thesis are written in *italics* and will be further introduced below. Firstly, the HPA axis, and perceived stress are in focus. Thereafter is the impact of comorbidities, perceived stress, and sense of coherence on self-rated health and health care utilization of IBS patients. Finally, the extraintestinal symptom of fatigue and its association to immune activation, central processes, and early adverse life events (EALs) are highlighted.

Figure 1. IBS-Biopsychosocial Conceptual model of pathogenesis and clinical expression, showing relationships between psychosocial factors, physiology, IBS symptoms and behavior as well as outcome factors. *Italic text indicates variables considered in this thesis.* ANS= autonomic nervous system, CNS= the central nervous system, HPA-axis= Hypthalamic- pituitary- adrenal axis, ENS= the enteric nervous system. Inspired and adapted from Drossman (15) and Tanaka (30).

Stress and HPA axis dysregulation

Exposure to stress is an important factor in the biopsychosocial concept in general, and in IBS in particular (36, 37). The biological underpinnings of

the hypothalamic-, pituitary-, adrenal- (HPA) axis and the sympathetic part of the autonomic nervous system (ANS), involved in the stress reaction, are thought to play important roles in the pathophysiology of IBS (36, 38, 39). Stressors such as early life trauma or chronic stressful life events experienced in adolescence and adulthood are considered major risk factors for IBS later in life (40). Further psychosocial stress of everyday life is thought of as a trigger factor for IBS alongside GI infections (36). The relationship between everyday perceived stress and gastrointestinal (GI) symptoms in manifest IBS, however, seems to be reciprocal rather than causal (41).

The stress concept

Stress is a somewhat troublesome term, since it has been so widely used during most of the last century, that some people argue that it has lost its utility (42). However, if well defined with clear biological terms anchored in health-related behavioral response, authorities of modern stress research still support the value of the concept, in understanding the processes and the multiple stages through which environmental adversities influence disease processes (43, 44).

The stress concept, as we know it, was initially described in 1936 as “the general adaptation syndrome” by the pioneering Hungarian-Canadian endocrinologist Hans Selye (45, 46). Since then, stress is thought of as an acute threat to the homeostasis of an organism. As a ubiquitous condition, it affects everyone, and it may be physical (for instance trauma or infection) or psychological (perceived) and may originate through events from the outside world or within. Importantly, stress evokes adaptive responses in several body systems, always defending the stability of the internal environment (homeostasis) to ensure the survival of the organism (45). Changes in these systems as a response to chronic stress have been referred to as allostatic load, a term initially invented and described in the 1990s by McEwen and Stellar (47, 48). This adaptation in response to potentially stressful threats engages activation of neural, neuroendocrine as well as neuroendocrine-immune mechanisms (49, 50).

Pathophysiological mechanisms of stress in IBS

The immediate response to stress includes activation of the HPA axis and the systemic sympathetic, and adrenomedullary (sympathetic) system (50-52). This can be generated by initial input from visceral or somatic afferents as well as cortical structures to a network comprised of integrative brain structures, mainly subregions of the hypothalamus, periaqueductal grey and amygdala. The integrative network further activates the sympathetic nervous system through the pontomedullary nuclei, and the HPA axis by

activation of paraventricular nucleus (PVN) in the hypothalamus. The activated PVN responds with secretion of corticotrophin-releasing factor (CRF) and arginine vasopressin. Those hormones activate the anterior pituitary gland to release adrenocorticotrophin (ACTH), which further stimulates the secretion of cortisol from the adrenal cortex. In the HPA axis there are also negative feed-back loops (50, 53). CRF and its receptors are primarily found in the brain, but also in the gut and are suggested to have a key role in stress-related alterations of GI function including stimulation of the colonic enteric nervous system and secretory motor function, increased permeability and visceral hypersensitivity of importance for IBS pathophysiology (54-57).

Initially activation of the stress system, leads to heightened arousal, accelerated motor reflexes, improved attention and cognitive function, increased tolerance of pain as well as decreased appetite and sexual arousal (50, 52). Further, it changes cardiovascular function as well as intermediary metabolism and inhibits immune activation and inflammation (51). Finally, cortisol gives negative feed-back to the PVN and hence down-regulates the stress response. The interaction between the integrative brain structures and the gut is schematic described in Figure 2. Note the involvement of both cortisol, as well as cytokines, both represented in these studies.

Figure 2. Pathways of interactions between integrative brain structures responding to stress and the gut. Adapted from Mayer 2000 (53).

Hair cortisol as a measure of long-term HPA axis activity

HPA axis activity and its final output; cortisol, can be determined by various methods including measures of concentrations in blood, saliva, and urine. Previous studies related to HPA axis activity in patients with IBS demonstrated conflicting results. Some studies reported higher morning levels of cortisol compared to controls (58-61), while others failed to find differences in basal cortisol levels (62, 63). There are also reports of higher cortisol values during the anticipation of stress in IBS patients compared to controls (64). A more sustained HPA axis response to an experimental psychosocial stressor as measured through cortisol in saliva has also been demonstrated in IBS (65) alongside a subdued response to a dexamethasone-test(62), both suggesting a suppressed HPA axis in IBS patients. In a meta-analysis from 2011 on 85 studies of HPA axis activity in functional somatic disorders, generally lowered levels of basal cortisol were seen in cases compared to controls. However this only reached statistical significance in chronic fatigue syndrome (66).

The measures of cortisol referred to above cover a relatively short time interval of HPA axis activity. Over the previous decades (since 2004) a method of measuring cortisol levels in hair has been developed (67-72). The method is considered valid, reliable and reproducible (73, 74). Hair cortisol concentrations (HCC) are described to reflect the amount of free, unbound cortisol (75). Since hair grows at an approximate rate of 0,35 mm per day, equivalent to approximately 1 cm per month, hair length represents an accurate index of average cortisol levels over time. It is hence possible to assess long-term cortisol exposure through the measure of HCC (76). An increasing number of cross-sectional studies support the association between HCC and different conditions with known associations to stress or HPA axis activity (77-83). Associations between HCC and different physical and psychological symptoms have been reported such as chronic pain (84) chronic fatigue syndrome (85) risk for myocardial infarction (86), depression and mental illness (71, 87) or the metabolic syndrome (88).

Previous associations between HCC and self-reported stress

Previous studies measuring the association between self-reported stress and HCC have demonstrated conflicting results. While some studies showed some associations (79, 89, 90), others demonstrated no or only weak associations between perceived stress and HCC (71, 72, 78). One study suggests that there could be a non-linear relationship with cortisol increasing with higher perceived stress, but dampened at the highest level (91). However, a meta-analysis conducted at the same time as the analyses of this thesis, somewhat surprisingly revealed no consistent associations

between self-reported stress and HCC. Further chronic stress, specifically in stress-exposed groups revealed a significant elevation in HCC when the stress was still ongoing while groups with absent/ past chronic stress showed a trend towards lower HCC (74).

Sense of coherence and coping

Beside perceived stress, another psychological aspect of importance for IBS and the biopsychosocial model is coping. It has been demonstrated that maladaptive coping and decreased self-perceived ability to reduce symptoms adversely affects health status in functional bowel disorders (92, 93).

In this thesis particularly, sense of coherence (SOC) is investigated in the context of associations between comorbidities and self-rated health as well as health care utilization. SOC is a theoretical construct explaining differences in how people perceive the world around them and thus how they tend to deal with stressful situations, i.e. coping capacity (94). The SOC concept includes three main components: comprehensibility (the ability to understand what is happening), manageability (the extent to which the person is able to manage the situation) and meaningfulness (the ability to find meaning in the situation) (95). Associations between IBS and reduced SOC was previously demonstrated by our group, and others (96-99).

Health care utilization

Costs for health resource utilization among patients with IBS in general practice is largely explained by comorbidity, which according to a Norwegian study generated ten times the costs when compared with individuals with IBS alone. In that study age, organic diseases and somatic symptoms were significant predictors of total costs, whereas IBS severity was not (100). However a Finish group has repeatedly described absence of associations between health care utilization and mental distress in IBS (101, 102). In a Danish study mental vulnerability in IBS patients explained some, but not all, of the use of healthcare and social benefits (103). Further an extensive systematic review regarding health care utilization of patients with IBS and non-ulcer dyspepsia, suggested that symptom severity explains a small proportion of health care seeking behavior. However psychosocial factors such as event stress, psychological comorbidity, personality, abuse and abnormal illness attitudes were more characteristic of those that sought help (104). A Swedish study of non-consulters as well as IBS patients of primary and secondary/ tertiary care revealed that mental health as well as poor social, emotional and physical functioning independently predicted health-care seeking (105). In paper II of this thesis, factors associated with having many health care contacts for all kinds of reasons, and not only regarding GI complaints, are investigated.

Self-rated health

Self-rating of over-all health is regarded as a consistent and powerful, independent predictor of health outcomes such as mortality, morbidity, disability and health care use (106). It should be differentiated from health-rated quality of life (107, 108), which is more often studied in IBS patients. It has however been demonstrated that IBS patients in general have poorer self-rated health than individuals not experiencing functional GI conditions (109). Low self-rated health in IBS has also been associated with presence of extraintestinal complaints and impaired psychosocial functioning, with the severity of GI symptoms playing a minor role (110).

Comorbidities of IBS

Excess comorbidity is present in a subset of IBS patients (111). A systematic review carried out 2008 revealed that among the extraintestinal comorbidities, fibromyalgia, chronic fatigue syndrome and chronic pelvic pain were the best documented (112). Psychiatric disorders such as major depression, anxiety and somatoform disorders have also been identified as common comorbidities of IBS and also a cause for people to seek health care for GI symptoms (113-115). However according to an American study of a little over 3000 IBS patients, no unique associations with certain other diagnoses were seen. In the subset of IBS patients with excess comorbidity in that study, not only symptom-based, but also biomarker-based diagnoses such as infections, osteoarthritis and stroke were more common, perhaps suggesting a general amplification of symptom reporting and physician consultation rather than associations with a few specific conditions (111). IBS patients with more comorbidities have also reported worse quality of life than those with fewer (116). Specific dyads of comorbidities (generalized anxiety, depression, back pain, agoraphobia, tension headache, and insomnia) have been associated with more severe symptoms of IBS, and pain (116).

Immune activation

Immune activation, low-grade inflammation or so called systemic chronic inflammation has generated a lot of interest during the last several years and inflammatory processes are thought to be involved in a wide variety of mental and physical health problems of today (117-119). Below are descriptions of both the putative involvement of immune activation in IBS pathophysiology, as well as its associations to the symptom of fatigue as part of a so-called sickness behavior.

Immune activation in IBS

IBS is not an inflammatory disease as such, but growing evidence suggests that dysregulation in immune function is associated with at least a subgroup of IBS patients and could contribute to either etiology or symptoms (21, 120-125).

This concept is supported by an established increased risk of developing IBS after infectious gastroenteritis (126-130) and also by GI symptoms in patients with inactive inflammatory bowel disease (131-133). Regarding postinfectious-IBS, in several studies, including a systematic review, psychiatric comorbidity or the occurrence of stressful life events around the time of the GI infection have been independent predictors of risk for IBS development (134-136).

Increased innate immune activity, particularly mast cells and monocytes, in both blood and intestinal mucosa has been described in a subset of IBS patients. There is also evidence for an activated adaptive response of the intestinal immune system possibly related to increased epithelial barrier permeability and an abnormal gut microbiota (120).

Cytokines

Communication between the different inflammatory cells as well as other cells involved in an immune response is coordinated by a diverse family of small glycoproteins, called cytokines. They influence both innate and adaptive immune responses, and can be classified as either pro-inflammatory (including interleukin (IL)-1, tumor necrosis factor- α (TNF- α), γ -interferon (IFN)- γ , IL-12, IL-18 and granulocyte-macrophage colony stimulating factor) or anti-inflammatory (including IL4, IL-10, IL-13, IFN- α and transforming growth factor-beta) (137-139).

This thesis particularly focuses on the associations of the pro-inflammatory cytokine TNF- α as an indication of immune activation and its association to the extraintestinal symptom fatigue. TNF- α is mainly secreted by activated macrophages as part of the innate immune response to pathogens. But it can also be produced and secreted by many other cell types such as mast cells, neutrophils, natural killer cells, T cells, eosinophils, neurons, and microglia (139, 140).

The findings of three previous studies on the association between immune activation and relations to fatigue or other psychological symptoms in IBS are presented in Table 2. Vara et al, found no associations between immune activation and fatigue in IBS (141). Others however point out an association between elevated TNF- α and other psychological symptoms tangent to fatigue or extraintestinal comorbidity, including chronic fatigue syndrome (122, 142).

Table 1. Associations between cytokines and psychological symptoms in IBS

Study	Population	Cytokines	Localisation	Relation to fatigue or other psychological symptoms/ comorbidity
Vara, 2018 (141)	All IBS subtypes, both genders	↑ TNF- α , IL-5, IL-6, IL-10 ↓ IL-1 β	Serum	No association to fatigue (FIS scores >25)
Scully, 2010 (142)	Female IBS patients, subtype not specified	↑ IL-6, IL-8, TNF- α , IL-1 β → IL-10, IL-12p70, IL-13, IFN- γ	Plasma	TNF- α and IL-1 β were additionally elevated in IBS patients with extraintestinal comorbidity including chronic fatigue syndrome.
Liebrechts, 2007 (122)	All IBS subtypes, both gender	↑ TNF- α , IL-1 β , IL-6	Peripheral blood mononuclear cell isolation – baseline and LPS stimulated	LPS induced TNF- α production in IBS patients was associated with HADS scores of anxiety.

IL= interleukin, TNF= tumor necrosis factor, FIS=Fatigue impact scale, LPS=lipopolysaccharid, HADS= hospital anxiety and depression scale.

Fatigue, sickness behavior and central correlates

Fatigue is one of the most common and disturbing extraintestinal symptoms of IBS (143-145). In a recent meta-analysis, 54 % of the IBS patients perceive fatigue (144) in accordance with a previous systematic review, which reported chronic fatigue syndrome in 51 % of the IBS patients (145). Several previous studies have also shown that experience of fatigue in IBS is related to anxiety, depression, and intestinal symptom severity (144, 146, 147).

Although fatigue is well recognized in IBS, the specific pathophysiologic mechanisms remain elusive. Fatigue is also a highly prevalent and persistent symptom of other pain syndromes (148). Alongside chronic widespread pain, fatigue in addition to unrefreshing sleep, cognitive difficulties and affective symptoms are for instance cardinal symptoms of fibromyalgia (149).

Previous research suggests that symptoms in, for instance, chronic pain disorders, depression and chronic fatigue syndrome could partly be due to a behavioral repertoire in response to inflammation (150-153). This is referred to as sickness behavior and has developed throughout evolution with the purpose to save energy for the sake of fighting infections. Sickness behavior presents similarly in animals and humans and includes fatigue, increased pain sensitivity, fever, malaise, nausea, anorexia, lethargy, depressed mood, anhedonia, increased anxiety, changed sleep patterns, decreased sexual activity and decreased movement (153, 154).

The central correlates of fatigue have been extensively researched in *e.g.*, multiple sclerosis (155-158), Parkinson's disease (159, 160), and chronic fatigue syndrome (161, 162). In these studies, several neural circuits have been associated with fatigue, of which the mesocorticolimbic circuit and its relation to dopamine function imbalance holds a prominent focus (163). In the mesocorticolimbic circuit, the nucleus accumbens (NAc) in the ventral striatum (164) is reported as being essential for the motivational dimension of fatigue (165), while the anterior cingulate cortex (ACC) and the prefrontal cortex link to the cognitive aspects of fatigue (165-167). Finally a physical dimension with the involvement in the perception of interoceptive signals is supposed to be represented by the anterior insular cortex (151, 168-170).

Within the mesocorticolimbic system immune activation and proinflammatory cytokines have been demonstrated to inhibit the synthesis, activity, and expression of neurotransmitters such as dopamine and serotonin (171-173).

Previous research also suggests that alterations in functional activity within these brain regions are associated with fatigue (165). Changes in the connectivity between different regions might also play an important role, but studies are scarce (165, 174-176). However, one recent study, in multiple sclerosis related fatigue, suggested altered resting state connectivity between specific subregions of the mesocorticolimbic network suggesting the ventral striatum (NAc) as a key hub (177).

As far as we know, central correlates of fatigue have not previously been studied in IBS. To further understand the analyzes of paper III in this thesis, principles of resting-state functional magnetic resonance imaging (rsfMRI), are described below.

Resting-state functional magnetic resonance imaging

The phenomenon of nuclear magnetic resonance has been known since 1938. In the 1970s this was coupled with advances in image acquisition methods, and since the 1980s magnetic resonance imaging (MRI) has been incorporated in clinical practices. In brief, MRI creates images of biological tissue through the utilization of strong magnetic fields. With these images, grey and white matter density, as well as cortical thickness of the whole brain or specific regions, can be visualized, measured and statistically compared (178). Much of our current knowledge regarding the brain is derived from these kinds of structural images. Such measurements, can however, not detect short-term physiological changes.

In the 1990s there was a breakthrough in research regarding brain function, when it was discovered that blood oxygenation in real-time could

be measured by this method, ushering a new era of functional MRI (fMRI) (178).

For a long time it has been known that oxygenated hemoglobin is diamagnetic and that deoxygenated hemoglobin has four unpaired electrons making it paramagnetic (179). It is also known that neurons do not contain any internal reserves of energy, and when activated they are supplied with increased regional blood-flow by what has been called a “hemodynamic response” (180, 181). It is this hemodynamic response that constitutes the base for fMRI.

Blood-oxygen-level dependent (BOLD) contrast can be detected when neural activity causes an increase in oxygenated blood flow to that area. Hence, the study of the brain function or “functional connectivity” shows patterns of simultaneous neuronal activity in, what can be, anatomically separated brain regions, thereby “connecting” these regions in terms of their co-activity (178, 181).

Furthermore, specific functional networks can be determined through the detection of different brain regions with synchronized neural activity in task-based studies (182). In IBS, it has been demonstrated that patients have altered responses to experimentally induced pain, often through rectal distention, involving brain regions of visceral afferent processing, emotional arousal and endogenous pain modulation (25, 182).

Even during rest, when the patient is asked to relax and to not think about anything in particular, fMRI can detect slow fluctuations in the blood-oxygen-level dependent (BOLD) signal in functionally connected brain regions. Resting-state fMRI (rsfMRI) was first described in 1995 (183) and is now widely used to determine function of specific brain regions as well as functional connectivity between different anatomically separated areas (184-187).

There are many ways of processing resting-state fMRI data, partly depending on the research question (187, 188). Using a data-driven approach our group has previously found associations between visceral sensitivity and alterations in functional connectivity within resting-state networks associated with interoception, salience and sensory processing (189). Also somewhat surprisingly, our group found that less colonic permeability correlated with more severe IBS symptoms as well as increased resting-state connectivity and structural connectivity in endogenous pain facilitation regions (190). In this thesis, we explore resting-state connectivity between regions of a mesocorticolimbic network in relation to fatigue.

Rationale for the thesis

IBS is a common disease in primary health care seriously affecting everyday life, but still the pathophysiology is not yet completely clear and probably varies between individuals. It is known that both extraintestinal symptoms, such as fatigue, as well as comorbidities contribute to the illness burden of IBS. A good doctor-patient relationship is important for treatment outcome (26). To fully understand the patient, it is of importance to consider the aspects of both underlying biological pathophysiological mechanisms as well as the influence of psychological and sociological components. In these studies, we focus on stress and the HPA axis, the impact of comorbidities and psychosocial factors on health outcome and finally mechanisms of the brain-gut axis related to fatigue. Through exploring these biopsychosocial mechanisms of IBS, this thesis will add to better understanding and care of IBS patients.

AIMS OF THE THESIS

The general aim of this thesis was to gain deeper understanding of the biopsychosocial mechanisms of IBS and its associations with stress, comorbidities, and fatigue, as reflected in the following specific aims:

- To compare levels of HCC as well as perceived stress between IBS patients and other primary care patients.
- To investigate whether HCC (a measure of biological stress) correlate with perceived stress in IBS and non-IBS patients.
- To evaluate the unique associations of comorbidities, GI symptoms, perceived stress, and sense of coherence with health care utilization and self-rated health in IBS patients and a control group of other patients.
- To elucidate fatigue and its association with circulating levels of a pro-inflammatory cytokine (TNF- α) in IBS and HC, and to explore the possible role of a resting state network of mesocorticolimbic regions known to be related to fatigue.

METHOD

This thesis relies on two large studies – the Twin cities IBS (TWIBS) study and the Brain-Gut study. Both use a cross-sectional design. The TWIBS study was an epidemiological investigation, also measuring HCC, conducted in a primary care setting. The Brain-Gut study was performed in a secondary care population with focus on mechanisms of brain-gut interactions. Both studies and the specific measures therein, used for this thesis, are described below.

Twin Cities IBS

Study I and II in this thesis are based on the case-control study Twin Cities. Ten Primary Health Care (PHC) centers, in the three major cities of the county of Östergötland, in south-east Sweden, joined the study. These PHC centers are responsible for a population of around 150 000 persons (about 1/3 of the region). The particular PHC centers were selected to ensure diversity concerning age-span, socio-economic factors, and number of immigrants.

Also the settings were such that one out of the three cities, could be labeled as a white-collar city (academic) and two as blue-collar cities (industrial) (191, 192). Two of these cities “the twin cities” Linköping and Norrköping, have previously been studied regarding public health problems, including GI diseases.

In general, these cities differ in a remarkable number of public health indicators such as prevalence of ischemic heart disease, sick leave, life-style factors and life expectancy, all in favour of the academic “white collar” city (192, 193). Regarding GI disorders; functional GI diseases and peptic-ulcer disease were more common in the white-collar city, while diseases of the gall-bladder and alcohol-related liver-disease were more common in the blue-collar city(191). The twin city aspect of this population is only considered for study I.

Recruitment in the TWIBS study

Recruitment was conducted between March 2010 and February 2014. A flow-chart of the inclusions and exclusions of study I and II of this thesis are presented in Figure 3. IBS-patients within the normal working age

range (18-65 years) with a clinical IBS diagnosis identified with ICD-10 diagnoses in the medical registers of the selected PHC centers were invited by mail to participate in the study.

Potential controls were identified as other patients at the selected PHC centers. They had no earlier GI diagnoses found in the patient register for the previous two years, were similar in terms of sex and age and had sought care for other complaints not associated with GI symptoms. After agreement to participate, the study candidate was given an appointment at the PHC center, where trained staff (myself included) cut a hair sample and handed out questionnaires alongside a pre-paid envelop to return. All IBS patients that were included reported active symptoms during the 2 years prior to inclusion.

Inclusion-, and exclusion processes for study I and II

The IBS-status of the study population for study I and II has been thoroughly investigated repeatedly using different approaches. Firstly, IBS patients were identified in the medical registres of the PHC centers as described above. GI disease was likewise used as exclusion criteria for the group of control patients identified in the primary care registry and was also asked for at recruitment.

Further, Rome III questionnaires were filled out by both IBS patients and non-IBS patients. (It is previously known that the Rome criteria are rarely used by primary care physicians (194).)

Answers to the Rome-criteria questionnaire as well as the reported medication or comorbidities revealed missed underlying GI problems in a few non-IBS patients. The patient records of these individuals were reviewed and 6 of these individuals turned out to have other GI diagnoses, and 9 persons actually had an IBS diagnosis, which had not been detected during the selection process. Four of those also full-filled Rome criteria and were hence shifted to the IBS-group. The other five were excluded. See Figure 3.

The exclusion criteria were further somewhat different depending on the planned investigations for study I and II. Drop-outs and exclusions of the 2 studies are fully presented in Figure 3. In total 186 IBS-patients and 360 non-IBS patients were available for final analyses of paper II and further when exclusions due to HCC analyses also were made 169 IBS-patients alongside 316 non-IBS patients were available for final analyses of study I.

Figure 3. Flow-chart of dropouts and exclusions for study I and II

The Brain-Gut study

Study III of this thesis is based upon the Brain-Gut study carried out at the Gastroenterology Department, University Hospital in Linköping. IBS patients aged 18-60 years were screened for eligibility to attend, including Rome III criteria. An overview of the inclusion process is presented in Figure 4.

All 88 included IBS patients fulfilled Rome III criteria, the prevailing version, at the time of recruitment (19), and none of the participants reported a history of gastroenteritis.

Forty-seven healthy controls (HC) without a medical history of GI symptoms or complaints were recruited by advertisement and received a monetary compensation.

Exclusion criteria for both study groups were any organic GI disease, metabolic or neurological disorders, and severe psychiatric disease (e.g. schizophrenia, bipolar disorder, psychosis) as well as nicotine intake within the last 2 months and inability to speak Swedish fluently. Further, a standard screening was performed to ensure no contraindications for MR scanning (e.g. metallic or electrical implants, history of claustrophobia, pregnancy etc.).

Figure 4. Overview of inclusions for paper III

Biochemical measures

Hair cortisol concentration

HCC were investigated in study I. All analyses were performed at the laboratory of clinical chemistry at the University of Linköping according to a method developed and validated in-house, using a competitive radioimmunoassay in methanol extracts (78).

At inclusion of the participants at the different PHC centers, trained staff cut approximately 100 strands of hair from the posterior vertex area of each of the participants' heads. This is in accordance with guidelines published by the Society of Hair Testing (195). Further, the hair was enclosed in identification marked and sealed plastic packages and stored in room temperature until analysis. The first 3 cm of outgrowth were analyzed. At least 3 mg of hair was required for reliable measurements.

Each sample was put into a 2 ml QiaGenRB sample tube with a 0,5mm QiaGen stainless steel bead. The samples were weighed on Sartorius MC 210p microscale and homogenized using a Retch Tissue Lyzer II (20 HZ). The sample tubes were placed in aluminium cylinders and frozen in liquid nitrogen for 2 minutes and the hair samples were thereafter homogenized for 2 minutes, producing fine hair powder. 1 mL of methanol was added to each tube and the samples were extracted for 24 hours on a moving board to keep the steel balls within the tubes in constant soft motion. 0,8 mL of the methanol supernatant was thereafter pipetted off and lyophilized using a Savant Speed Vas Plus SC210A.

Finally, the samples were dissolved in a radioimmunoassay buffer. The specific primary antibody and I-125 (radioactive iodine) was added and incubated for 48 hours. The process continued with secondary antibody and centrifuging before the samples were counted in a gamma counter.

TNF- α

Plasma levels of TNF- α , was measured in study III and elevated levels could be regarded as a sign of immune activation. For reasons of feasibility, this was accomplished within a mean of 9 days (1-24) after the MR scan for IBS patients and within a mean of 6 days (1-14) for controls. When looking back into the booking schedule, no systematic differences emerged between groups with respect to the time of the day when blood was drawn (data not shown).

The participants were instructed to refrain from the use of anti-inflammatory drugs within 24 hours prior to blood draw. Six milliliters of fasting venous blood samples were collected in EDTA-treated tubes. To each milliliter of blood, a solution of 1.3 mg EDTA and 50 ml Trasylol 10 000 KiE was added. Blood samples were centrifuged (15 min, 3400g) at 4°C, and plasma was redrawn and frozen at -80°C until analysis. An ultrasensitive human TNF- α ELISA kit was used according to the manufacturer's instructions. Undiluted plasma samples, controls (positive and negative), and standard point samples were added in duplicates to the plate. Absorbance was measured at 450 nm in a VERSAmax Tunable Microplate Reader (Molecular Devices, CA, USA) using Softmax pro 5 (Molecular Devices).

Questionnaires

Several validated questionnaires were used for different purposes in the separate studies, described below. These represent key constructs which cannot be measured through objective means. An overview is given in Table 2.

Table 2. Overview of the questionnaires used in each study of the thesis

Questionnaire	Study I	Study II	Study III
Rome III criteria	x	x	x
PSS	x		
Self-reported health		x	
GI symptom diary		x	
SOC questionnaire-13		x	
mFIS			x
IBS-SSS			x
HADS			x
Mc Arthur			x
CTQ			x

PSS= perceived stress scale-14, GI= gastrointestinal, SOC= sense of coherence, mFIS= modified fatigue impact scale, IBS-SSS= IBS severity scoring system, HADS= hospital anxiety and depression scale, MacArthur= MacArthur scale of subjective social status, CTQ= childhood trauma questionnaire.

Rome criteria

In both the TWIBS- and the Brain-Gut study all participants completed a questionnaire that enabled evaluation of the Rome III criteria. Please see introduction for background and details regarding these criteria.

Perceived stress

To measure perceived stress in studies I and II, we used the Perceived Stress Scale-14 (PSS) (196). The scale measures the degree to which the respondent has considered their life to be unpredictable, uncontrollable and/or overloaded during the last month. The respondents were asked how often they felt a certain way on a 5-point ordinal scale coded: never=0, almost never=1, sometimes=2, fairly often=3 and very often=4. In the scoring algorithm, positively worded items are reverse coded, consistent with recommended scoring methods (196). The Swedish version, as well as the original English, has been demonstrated as reliable and valid [Eskin M,

Parr D. Introducing a Swedish version of an instrument measuring mental stress: Reports from the Department of Psychology, University of Stockholm. 1996:813].

Self-reported health

For the TWIBS study the participants were asked a five point-scale question about general health derived from The Swedish Living Conditions Survey of Health and Welfare [Statistics-Sweden. Employment, working hours and work environment 1994-95. Sveriges Officiella Statistik. Stockholm: Statistiska Centralbyrån (SCB), 1998.(In Swedish)]. The ratings were: 1=excellent, 2=very good, 3=good, 4=fairly good, 5=bad. For the analyses of paper II this was dichotomized into 1-3= good health and 4-5=poor health. The Likert scale of self-rated health is commonly collapsed into a dichotomous variable of good versus less than good health (109, 197). Similar results regarding size and significance of main effects, type of association and interaction effects have been found when using a logistic regression model with that dichotomous variable as when using alternative statistical methods which incorporate the original ordinal scale of self-rated health (197).

The GI symptom diary

GI symptoms were collected prospectively and analyzed for study II. The study participants recorded their GI symptoms in a validated (198) symptom diary over a period of two weeks. For each day (24 hours), they recorded the episodes of abdominal pain and bloating and graded the pain intensity into light (1), moderate (2) or intense (3). Further calculations of diary data for study II gave average days and hours of abdominal pain per week, as well as bloating per week. As about 22% of the total reported pain intensity in the TWIBS study was incorrectly reported (higher than maximum), the variable "intensity of pain" was omitted from the analyses as potentially unreliable. The other measures in the diary did not include ratings; hence, no similar mistakes could have been made by the participants.

Sense of coherence

The Swedish version of Antonovsky's 13-item sense of coherence questionnaire (SOC-13) (199) was used as a measure of SOC and indirectly also as a measure of coping in paper II. Every item is scored on a Likert scale ranging from 1 to 7 points. Thus, the total score has a range of 13–91 points. The higher the score, the more effective the coping strategy. This 13-item version of SOC has been shown to be reliable, valid and cross-culturally

applicable when evaluating how people can manage stress and still be healthy (200, 201).

IBS-SSS

The IBS Severity Scoring System was used for rating IBS symptoms in paper III. The scoring system incorporates five items: abdominal pain severity, pain frequency, bowel distension, bowel dysfunction and quality of life/global well-being. Total maximum score is 500. Mild, moderate and severe symptom burden are indicated by scores of 75–175, 175–300 and > 300 respectively (202).

HADS

Anxiety and depression in paper III were assessed using the Hospital Anxiety and Depression Scale (HADS) (203). Summary scores ranging from 0 to 21 indicate increasing severity of current symptoms of anxiety and depression, respectively. HADS scores 8–10 indicate that anxiety/ depression could be present, while scores above 10 indicate that anxiety/ depression is very likely present.

Modified fatigue impact scale

Fatigue is the key construct of study III. There are different ways of measuring fatigue. One way is to measure the impact of perceived fatigue in daily life. In this thesis the modified version of the Fatigue Impact Scale (mFIS) is used (204–206).

In the mFIS questionnaire, fatigue is defined as “a feeling of physical tiredness and lack of energy that many people experience from time to time”. The participants were asked to rate how much their experience of fatigue have influenced physical, cognitive, and psychosocial dimensions in their daily life during the last 4 weeks. The mFIS questionnaire contains 21 questions and responses were made on a five-point scale where 0 = never, 1 = rarely, 2 = sometimes, 3 = often, and 4 = almost always, generating a total MFIS score range of 0–84. The questionnaire was developed for MS patients but have also been validated to use in the research of gastrointestinal disease (206).

MacArthur scale of subjective social status (SES ladder)

For background characteristics in paper III, the MacArthur Scale of Subjective Social Status was used to assess the individual’s sense of social status, which is tightly linked to health status and psychological functioning (207, 208). A summative measure of the subjective social status is provided using a symbolic stepladder image.

Childhood trauma questionnaire

Early adverse life events were assessed for additional analyses, not included in the paper, of study III, regarding associations with fatigue and immune activation. The Childhood trauma questionnaire (CTQ) (209) is used for retrospective recall of five forms of maltreatment – emotional, physical, and sexual abuse, and emotional and physical neglect. Items are rated on a 5-point Likert-type scale, with response options ranging from “Never true” to “Very often true” with sum scores rating from 5 to 25 respectively.

Data from the regional healthcare registry

Comorbidities and number of PHC contacts for the analyses of paper II, were obtained through the regional healthcare registry (210). This registry is based on a personal code linked to birth date and gender for all the inhabitants of the region. The database comprises all diagnoses from primary care, hospital outpatient and hospital inpatient care. Alongside the total number of unique diagnoses for each participant between 2010 and 2014, the presence of extra-intestinal pain (ICD-10 codes M79, M54, R52, R51, G44, G43, N94, N80), depression (ICD-10 code F3), anxiety (ICD-10 code F4) and sleeping disturbances (ICD-10 code F51) were identified. Numbers of primary healthcare contacts during this five-year period were also extracted from the same registry.

RsfMRI procedures

RsfMRI was measured in the Brain-Gut study and analyzed in study III of this thesis.

All participants were requested to fast for at least four hours (water was acceptable) and to refrain from alcohol consumption and from taking any medications for pain, sleep, or IBS symptoms for at least 24 h prior to scanning.

MRI was performed on a 3T Philips Ingenia (Philips Healthcare, Best, The Netherlands) located at the Center for Medical Image Science and Visualization (CMIV) at Linköping University, Sweden using a 32-channel head coil. Participants were instructed to rest with their eyes closed during the 10 minutes long fMRI scan. Whole-brain rsfMRI data were acquired with a single-shot, gradient-echo echo-planar imaging (EPI) sequence with repetition time, TR = 2000 ms; echo time, TE = 37ms; voxel size = 3.59x3.59x4 mm³; number of slices = 28; SENSE factor = 2. In addition, T1-weighted (T1W) images were acquired for all participants to ensure that they were otherwise free from any obvious pathologic abnormalities.

Analyses of rsfMRI

RsfMRI data were thereafter preprocessed and analyzed using the CONN functional connectivity toolbox (211, 212). Preprocessing was performed using the standard pipeline in CONN with realignment and unwarping for estimation and correction of subject motion; slice timing correction, ART-based outlier detection and scrubbing for the removal of signal intensity and motion artefacts; segmentation of the T1W images into grey matter, white matter, and cerebrospinal fluid as a preparation for normalization of the functional images to Montreal Neurological Institute (MNI) coordinates; smoothing using a Gaussian kernel of 8 mm for spatial convolution to ameliorate inter-subject differences in anatomy. To denoise the functional images, a band-pass filter of 0.008–0.09 Hz and linear detrending were used. Functional connectivity was calculated as bivariate correlation with hemodynamic response weighting.

Functional connectivity between specific regions of interest (ROIs) was investigated by ROI-to-ROI analysis using the CONN toolbox. Here, we specifically investigated regions of the mesocorticolimbic network known to be related to cognitive, motivational, and physical aspects of fatigue (165). This was done using CONN default masks for bilateral NAc, bilateral middle frontal gyrus (here representing the dorsolateral prefrontal cortex, DLPFC), and the ACC. Because several previous studies indicate the anterior insula as an important hub for fatigue, while discussing the insula without describing anatomical substructures (25, 116), we decided to use manually segmented masks for the anterior-middle insulae (amINS) in this study (25). In total, we investigated the resting-state functional connectivity between seven ROIs, as visualized in Figure 5.

Figure 5. Visualization of the regions of interest (ROI) used in the functional connectivity analysis. A) Dorsolateral prefrontal cortex (blue), anterior cingulate cortex (green), nucleus accumbens (yellow), left view. B) Dorsolateral prefrontal cortex (left side = purple, right side = blue), anterior cingulate cortex (green), nucleus accumbens (yellow), anterior view. C) Anterior and middle insula, left view.

Statistical analyses of rsfMRI data

Statistical analyses of rsfMRI data were performed using the CONN software (212).

Firstly, we confirmed that the selected ROIs comprised a functionally connected network in our total sample of IBS patients and HC using one-sample t-tests. We also compared the network's functional connectivity between IBS and HC using two-sample t-tests.

Secondly, associations between fatigue impact and inflammatory markers, respectively, and functional connectivity in this network were investigated by linear regression analysis using mFIS total scores or plasma levels of TNF- α for each participant as covariates of interest and HADS scores as covariates of non-interest, given that mood disorders could impact connectivity in IBS patients (24). The linear regression analyses were performed both within the IBS and HC groups separately, as well as between groups, in order to compare IBS with HC. A Fisher transformation (inverse hyperbolic tangent function) was applied to the correlation measures in order to improve the normality assumption before second level between-subjects analyses. Results from the functional connectivity analyses are reported with p-values that are false discovery rate (FDR) corrected for multiple comparisons, using a statistical threshold of $p_{\text{FDR}} < 0.05$.

Statistics

All statistical data analyses, except regarding rsfMRI statistics (described above), for this thesis were performed using SPSS software, versions 21, 22, 25 and 26 (SPSS Inc., Chicago, IL, USA).

Analyses of comparisons and correlations

All three studies contained some or several dependent or independent variables with non-normal distributions, which was taken into consideration in the analyses.

For paper II and III, since not all the numerical background variables were normally distributed, they were compared using the Mann-Whitney U-test and presented as medians and interquartile range.

Age, the only quantitative background variable in paper I, was normally distributed, and presented as mean and standard deviations, as well as compared between groups using the student's t-test. The outcome variable PSS-14, also had a normal distribution and the total scores were reported in means and standard deviations and compared between groups with student's t-test. The biological stress variable of paper I, HCC, on the other

hand were skewed towards higher values. Original values were presented as median and interquartile range. Due to the skewness, HCC were logarithmic transformed before further analysis, which de-emphasizes large outliers. However, the Shapiro-Wilk test did still not support the assumption of normally distributed residuals, which was taken into considerations regarding the regression analyzes described below. Due to the large sample size mean and standard deviations were presented for the logarithmic transformed HCC values, and differences between groups were calculated using student's t-test. For this framework report however a Mann-Whitney U-Test was also performed of the original HCC values.

For an alternate, illustrative view in paper I, we also calculated quintiles of hair cortisol i.e. the population studied divided into fifths (quintiles). Differences between IBS patients and controls with respect to the distribution of quintiles were analyzed using the chi-squared test.

The qui-squared test was also used for comparison of all other categorical variables of the three studies.

For paper I, Pearson's correlations between all the background variables and PSS-14, logarithmic HCC and quintiles of HCC were calculated mainly for description, while formal statistical inference of regression analyses were adjusted for non-normal distributions of logarithmic HCC, as described below. The correlation between logarithmic HCC and PSS-14 presented in this framework report is however calculated using Spearman's rank test.

In paper II and III, Spearman's rank test was also used for correlational analyses because of the non-normal distributions of some variables.

Regression analyses

In *study I*, potential confounders among all background variables, in the analysis of covariance of IBS and the three stress variables were controlled for via multiple linear regressions with a backward elimination approach. To adjust for the non-normal distribution of also logarithmic HCC, we used the nonparametric bootstrap for formal statistical inference. Unstandardized coefficients and standard errors were presented.

Study II had two categorical dependent variables - self-reported health and health care utilization. Self-reported health was dichotomized into good and poor, as described above in the methods section.

For the second dependent variable, IBS patients were divided into those with many PHC contacts (the upper quartile of the whole sample) and those with fewer contacts (the three remaining quartiles).

Univariate and multivariable logistic regressions reported as odds ratios with 95% confidence intervals were used to assess all the independent

variables as potential predictors of the probability of patients reporting: i) good health and ii) having many PHC contacts.

In the multivariable logistic regressions, only one GI symptom variable was included due to multicollinearity among the three possible variables. Because of substantial redundancy in the full multivariable models, model reduction was performed using a backward elimination method. Variables were removed if $p > 0.05$ at each step of the procedure.

The ability of the logistic regression models to discriminate between outcome categories was further assessed through the area under the Receiver Operator Characteristic (ROC) curves (AUC). AUC values close to 0.5 indicate no discrimination, while values close to 1.0 indicate perfect discrimination.

In study III multiple linear regression analyses were performed in the IBS group regarding the association between TNF- α and mFIS. A stepwise, backward approach was implemented, including mFIS, HADS, sex, age, BMI and psychopharmaceuticals. Potential predictive variables were removed one at a time if their p -value was > 0.05 . The Shapiro-Wilk test did not support the assumption of normally distributed residuals of TNF- α values in IBS ($p < 0.001$). To adjust for the non-normal distribution of TNF- α , we again used the non-parametric bootstrap (1000 replications) for formal statistical inference.

Multiple regression analyses were also performed in order to control for sex, age and mood disorders regarding the statistically significant correlations of mFIS and CTQ domains. The Shapiro-Wilk test did support the assumption of normally distributed residuals of mFIS in IBS patients, therefore regression analyses were made using conventional statistical inference.

Statistical significance

All tests throughout this thesis are two-tailed and statistical significance is set to $p < 0.05$.

Dropouts and missing data

A response bias analysis was made for paper I regarding dropouts, that did not complete the questionnaire or provide hair for analysis. This revealed that the non-responders were more frequently male and younger than the responders in both groups. Participants with missing values were excluded pairwise in all analyses.

Ten IBS patients and 9 controls who were included did not give complete answers to the PSS questionnaire. Only a small fraction of background variables was missing. That is $< 1\%$ in all variables except for

hormone medication (2,7%) and CNS-active medication (2,9%). There was no difference in age between responders of PSS and non-responders.

For the analyses of paper II number of responders are clearly indicated in Table 10 in the result section. No further dropout analyses were made.

For paper III data on TNF- α levels were obtained from all participants, whereas other outcome measures were not available from all patients and HC, resulting in missing data as follows: Two IBS patients did not complete the MRI part of the study and fMRI data from 4 IBS was excluded due to excessive motion. Four IBS patients did not fill-in the mFIS questionnaire, and HADS scores were missing from one patient. IBS-SSS was not completed by one IBS patient and one HC. The MacArthur Scale of Subjective Social Status was not filled in by five IBS patients. The body mass index (BMI) was not available for 16 IBS patients. In summary, it is unlikely that missing data has biased these analyses.

Considerations of multiple comparisons

Results from the functional connectivity analyses are reported with p-values that are false discovery rate (FDR) corrected for multiple comparisons, using a statistical threshold of $p_{FDR} < 0.05$.

In paper I and II no such corrections were made. As is common in epidemiological studies, we interpret the findings as providing evidence to support our hypotheses rather than definitively proving them to be correct. For this reason, we have not adjusted for the number of hypothesis tests performed.

Outliers

In both the TWIBS and the Brain-Gut study considerations were taken regarding a few outliers of the biological measures HCC and TNF- α . If no reason for exclusion was found when going through the medical records those participants were kept in the study in order not to manipulate data. However, precautions were taken due to non-normality as described above.

Power and sample size

Study I: A sample size calculation regarding HCC was made using Altman's nonogram. The power was set to 0.80 and statistical significance at 0.05 (two-tailed). Previous studies revealed a standardized difference of approximately 0.3 between exposed and not-exposed (79, 80), which yielded a total sample-size requirement across IBS cases and controls of approximately 350 individuals. Since our sample size exceeded that number, we concluded that it had adequate statistical power.

Study II: The available sample size of study II provided statistical power 0.8 at the 0.05 level of statistical significance (two-tailed) for a dichotomous discriminator with odds ratio 2.5 or greater or a quantitative discriminator with odds ratio 1.55 (per standard deviation change in the discriminator) or greater. Since those were moderate effect sizes, we concluded that the available sample size provided adequate power for the purposes of paper II.

Study III: Power and sample size calculation were conducted for the Brain-Gut study originally in order to determine differences in between the three different types of IBS (diarrhea, constipation or mixed-type). That was for other purposes than study III of this thesis and here only differences between IBS patients and HC are searched for. A survey of previous IBS neuroimaging literature for evidence of functional and structural brain differences related to these conditions indicated an average reported effect size of $d = 2.2$ for fMRI data. For the one-way three group fixed effects ANOVA framework with a proposed enrollment of 105 subjects (35 subjects per group), power calculations showed that it would be possible to confidently detect large, meaningful effects ($d > 0.6$) with a Greenhouse-Geisser corrected F at a Bonferonni-adjusted $\alpha > 0.005$ (to account for multiple regions-of-interest) with a confidence of $1-\beta \geq 0.8$. In this case with only 2 groups a sample size of less than 10 persons per group would have been enough. However, further secondary analyses regarding the multiple regressions of fMRI analyses with 3 independent variables and a presumed small effect size revealed that a population of 76 subjects would be needed to provide statistical power 0.8 at 0.05 level of statistical significance (two-tailed).

ETHICAL CONSIDERATIONS

Ethical approval for both the TWIBS and the Brain-Gut Study were obtained from the Regional Ethical Review board in Linköping, Sweden (Dnr M41-09 (TWIBS) and Dnrs 2013/506-32; 2014/264-32 (the Brain-Gut study)).

All participants of both studies gave written, informed consent according to the Helsinki declaration. All data were deidentified, aggregated and presented at a group level. Participants of the TWIBS-study were also specifically asked for permission regarding data to be obtained from government registries. They could also leave the study if they chose to at any point without consequence. If a participant chose to withdraw consent all their data were destroyed including questionnaires biological samples.

Regarding study procedures, the participants of the Brain-Gut Study underwent an MR scan. To avoid experiences of claustrophobia or anxiety during the scan, subjects with pre-existing claustrophobia were excluded. The medical and research staff were experienced in these settings and well able to assist subjects with any distress. Subjects were also able to terminate the experimental procedure whenever they wanted, by pressing a patient alarm button.

RESULTS

Characteristics of the two study populations

General background characteristics available in both the TWIBS and the Brain-Gut study populations are presented in Table 3. In both populations a clear female predominance was seen. That is in line with, but somewhat more pronounced than, what is generally reported in IBS. For the TWIBS population comorbidities including mood disorders, were self-reported in study I, but also extracted from the regional health care registry for the analyses of study II. In the Brain-Gut study (study III of this thesis) possible comorbidity of anxiety and depression was captured by the HADS questionnaire. Even though these measures of mood disorders are not quite comparable, the secondary care population seems more burdened by mood disorders than the patients in the primary care study. In both study populations mood disorders were substantially more common among IBS patients than in the control groups (Table 3). Further study population characteristics that are relevant for the specific analyses of study I-III are presented in the following sections.

Further sociodemographic and clinical characteristics of the TWIBS population (study I and II)

Despite the intention of matching, IBS patients were somewhat younger than non-IBS patients in paper I and II (Table 3). Regarding gender and psychosocial environment (living in a blue-, or white-collar town) the matching was more successful. Alongside having more psychiatric comorbidities, described above, IBS patients were also more likely to report extraintestinal pain disorders, and sleeping disturbances than non-IBS patients (Table 4). That is also consistent with data obtained from the health care registries for paper II (Table 5) Self-reports of cardiovascular disease, hypertonia and diabetes were similar in both groups. Analyzes for paper I further showed similar smoking habits as well as education levels in the two groups, while more non-IBS patients were born in Sweden and worked full-time (Table 4)

Comorbidity in terms of given diagnoses, self-rated health and health care utilization is further investigated in paper II (Table 5). IBS patients reported poorer health and had more than the double amount of PHC contacts compared with non-IBS patients. Perceived stress, which is investigated in both paper I and II is consistently higher in the IBS population of the TWIBS study (table 4 and 5). Paper II further demonstrates lower sense

of coherence, and (as expected) more GI symptoms in the IBS group (Table 5).

Table 3. Background characteristics of the two study populations

	The TWIBS study				The Brain-Gut study	
	Study I		Study II		Study III	
	IBS	Non-IBS	IBS	Non-IBS	IBS	HC
N	169	316	186	360	88	47
Age, median (IQR)	48.0 (25)	54.0 (17)	48.0 (24)	54.0 (17)	30 (11)	31 (19)
Female gender % (n)	86% (146)	85% (269)	80% (149)	83% (299)	85% (75)	85% (40)
Psychiatric comorbidity % (n) :	23% (39)	5% (17)				
Depression diagnosis, % (n)			25% (7)	10% (35)		
Anxiety diagnoses, % (n)			38% (70)	16 % (58)		
HADS Depression 8-10, % (n)					16% (14)	0% (0)
HADS Depression >10, % (n)					14% (12)	0% (0)
HADS Anxiety 8-10, % (n)					25% (22)	11% (5)
HADS Anxiety >10, % (n)					44% (39)	4% (2)
Self-reported CNS active medication, % (n)	37% (59)	16% (51)				
Prescribed psychopharmaceuticals, % (n)					38% (33)	0% (0)
Subtype of IBS (paper I and III), % (n) :						
IBS-C	12% (21)				18% (16)	
IBS-D	27% (46)				28% (25)	
IBS-M	51% (87)				53% (47)	
IBS-U	9% (15)				-	

TWIBS = Twin Cities IBS study, HC= Healthy Controls, IQR= Interquartile range, HADS=Hospital anxiety and depression scale, CNS= central nervous system, IBS-C= IBS with predominantly constipation, IBS-D= IBS with predominantly diarrhea, IBS-M= IBS with mixed stool type manifestation. HADS scores 8-10: anxiety/ depression could be present. HADS scores>10: anxiety/ depression is very likely present.

Table 4. Further population characteristics for study I

	IBS	Non-IBS	
	% (n)	% (n)	p
Living alone/ with parents	29% (48)	17% (55)	0.005
Married de facto	72% (121)	83% (261)	
Living in a blue-collar city	56% (95)	54% (172)	0.707
Living in a white-collar city	44% (74)	46% (144)	
Employment status :			
Full-time work	37% (62)	52% (164)	0.005
Part-time work	24% (41)	21% (67)	
Unemployed	39% (65)	27% (85)	
Educational level :			
Low	15% (26)	9% (28)	0.059
Medium	46% (77)	44% (139)	
High	39% (66)	47% (147)	
Born in Sweden	82% (139)	92% (289)	0.002
Ever been a regular smoker	43% (73)	46% (146)	0.506
Self reported comorbidities (current) :			
Cardiovascular disease, hypertonia or diabetes	17% (28)	14% (46)	0.557
Other pain disorders	57% (96)	39% (126)	<0.001
Sleeping disturbances	37% (63)	19% (61)	<0.001
Psychiatric disease	23% (39)	5% (17)	<0.001
Steroid inhalations	9% (15)	7% (23)	0.533
HRT, oral contraceptives and tyroidea hormones	20% (32)	13% (39)	0.036
Severe life event during the last year	53% (89)	39% (122)	0.003

Categorical data is presented with percentages (counts within brackets) and compared between groups with a chi-squared-test. CNS= central nervous system, HRT= hormone replacement therapy.

Table 5. Further population characteristics for study II

	IBS patients (n=186)		Non IBS-patients (n=360)		p
	n	%(n) or median (IQR)	n	%(n) or median (IQR)	
PHC-contacts (2010-2014)	185	26 (25)	348	12.0 (15.0)	<0.01
Self-rated good health	183	59.7% (111)	360	90.8% (327)	<0.01
No comorbidities	185	14.0 (11)	346	8.0 (7)	<0.01
Depression	185	25.4% (47)	346	9.7% (35)	<0.01
Anxiety disorder	185	37.8% (70)	346	16.1% (58)	<0.01
Sleeping disorder	185	14.5% (27)	346	7.2% (26)	0.01
Pain disorders	185	70.8% (131)	346	50.0% (180)	<0.01
Perceived stress scale^a	178	25.0 (11)	352	21.0 (10)	<0.01
Sense of coherence scale^b	181	61,0 (18,0)	358	69,0 (15,0)	<0.01
Av. days abd. pain/ week	176	3.0 (3.5)	353	0.0 (0.5)	<0.01
Av. hrs abd. pain/ week	176	16.5 (34.0)	353	0.0 (1.0)	<0.01
Av. days bloating/ week	176	3.5 (4.0)	353	0.0 (0.52)	<0.01

Categorical data is presented with percentages (counts within brackets) and compared between groups with a chi-squared-test. Numerical data is presented with median and IQR and compared between groups with a Mann-Whitney U-test. PHC= primary health care, No= numbers of, Av.= average, hrs= hours, abd= abdominal. ^a The perceived stress scale has a possible range 0-56. ^b The sense of coherence scale has a possible range 13-91.

Further sociodemographic and clinical characteristics of the Brain-Gut study population (study III)

In the Brain-Gut-Study there were no differences regarding age, gender, or BMI between IBS patients and HC (Table 3).

The participants sense of social status was measured with the Mc Arthur ladder and showed lower values in the IBS group. As reported above IBS patients were more burdened with symptoms of anxiety and depression. IBS-SSS scores were higher in patients, as expected, and indicated moderate to severe symptoms in this group. Finally, IBS patients also reported more experience of all types of childhood trauma, measured with CTQ (Table 6).

Table 6. Further population characteristics of Study III

	IBS	HC	p
Socioeconomic status	6.0 (2)	7.0 (1)	<0.001
BMI	23.4 (5.2)	23.4 (17.1)	0.926
HADS, depression	5.0 (6.0)	1.0 (2.0)	<0.001
HADS, anxiety	10.0 (7.0)	3.0 (3.0)	<0.001
IBS-SSS	325.0 (119.0)	8.0 (27.0)	<0.001
CTQ Emotional abuse	8.3 (0.5)	5.9 (0.4)	<0.001
CTQ Physical abuse	5.9 (0.2)	5.0 (0.2)	0.007
CTQ Sexual abuse	5.9 (0.4)	4.7 (0.2)	0.009
CTQ Emotional neglect	9.0 (0.5)	6.9 (0.5)	0.007
CTQ Physical neglect	6.4 (0.3)	5.3 (0.3)	0.003

Data are given as a median and interquartile range in parentheses. Group comparisons were performed using the Mann-Whitney-U-test. IBS = Irritable bowel syndrome, HC = healthy controls, BMI = body mass index, HADS = Hospital Anxiety and Depression Scale, IBS-SSS = IBS Severity Scoring System, CTQ = Childhood Trauma questionnaire.

Paper I

Hair cortisol concentrations and perceived stress

HCC was lower and perceived stress was higher in IBS patients in comparison with the other primary care patients (Table 7). Likewise, there was also a statistically significant difference in the distribution of the quintiles of HCC between IBS and non-IBS patients. The highest proportion of IBS patients was found in the quintile of the lowest HCC. The opposite was seen in non-IBS patients with fewest participants in the first quintile (Fig. 6).

Table 7. Hair cortisol concentration and perceived stress			
Outcome measures	IBS patients	Non-IBS patients	p
HCC (pg/mg)	16.3 (26.9)	22.8 (29.1)	0.001
HCC _{log} (pg/mg)	3.0 (1.1)	3.3 (1.0)	0.022
PSS-14	25.3 (8.0)	21.4 (7.5)	<0.001

Original hair cortisol being not normally distributed is presented in median and IQR and compared between groups with the Mann-Whitney U-test. The log transformed hair cortisol and PSS-14 are presented with mean and s.d and compared between groups using student's t-test
PSS= perceived stress scale.

Figure 6. Quintiles of Hair Cortisol Concentrations.Distributions (percentage) of cortisol levels (pg/mg) divided into quintiles. 1. (low): 3,1-11,4 (median=8,00), 2: 11,5-16,2 (median=14,0), 3: 16,3-25,3 (median= 20,8), 4: 25,6-47,2 (median=32,4), 5 (high): 47,3-8520,5 (median=84,4).

Univariate and multivariable analyses of the stress variables

Descriptive, univariate correlations, and multivariable regression analyses between both logarithmic HCC, quintiles of HCC and PSS on the one hand and IBS as well as other relevant variables with potential associations to stress on the other, are presented in Table 8. Overall, only weak correlations and associations were seen.

IBS remained associated to both quintiles of HCC as well as PSS-14, but not logarithmic HCC, in the final models of backward elimination, which are presented in Table 8.

In the final regression model alongside IBS, female sex, the psychosocial environment, and non-smoking were negatively associated with levels of HCC.

Regarding perceived stress, having IBS, alongside psychiatric comorbidity as well as sleeping disorders were positively associated while age was negatively associated with HCC.

Only age, and sex stayed associated to logarithmic HCC in the final regression model of logarithmic HCC.

Table 8. Univariate and multivariable analyses of the stress variables

	Univariate correlations (r)			Multiple regressions (B (S.E.))		
	HCC _{log}	Quintiles of HCC	PSS-14	HCC _{log} ^a	Quintiles of HCC ^b	PSS-14 ^c
Irritable bowel syndrome	-0.107*	-0.146**	0.232**		-0.332 (0.146)*	1.647 (0.759)*
Age	0.115*	0.101*	-0.191**	0.08 (0.004)*		-0.089 (0.027)*
Female sex	-0.085	-0.103*	0.029	-0.271 (0.125)*	-0.418 (0.173)*	
Single	0.075	0.077	-0.080			
Living in a blue collar town	-0.047	-0.097*	-0.003		-0.304 (0.126)*	
Born in Sweden	-0.007	-0.000	-0.137**			
Employment status (Full-time, part-time, unemployed)	0.027	0.022	0.106*			
Educational status (low, medium, high)	-0.002	-0.012	-0.110*			
Never been a smoker	-0.104*	-0.115*	0.024		-0.342 (0.127)*	
Cardiovascular disease, hypertonia or diabetes	0.022	0.031	-0.044			
Other pain disorder	-0.017	-0.041	0.145**			
Psychiatric comorbidity	-0.111*	-0.124**	0.353**		-0.420 (0.197)*	6.975 (1.125)**
Sleep disturbances	-0.034	-0.052	0.209**			2.691 (0.811)*
Steroid inhalations	0.023	0.005	-0.037			
CNS-active medication	-0.034	-0.082	0.171**			
Hormone medication	-0.066	-0.041	0.070			
Severe life event the last year	-0.022	-0.005	0.122**			
				R ² =0.186	R ² =0.063	R ² =0.179

Pearson's correlation coefficients are presented. For the multivariable regression analyses, the models were selected through a backward elimination exercise. The values for the variables of the final models are presented. For quintiles of and logarithmic HCC, the unstandardized coefficients in the multivariable regressions were estimated through bootstrapping. * p<0,05, **p<0,001, ^adf=4, F=4,302, p=0,002, ^bdf=5, F=6,412, p<0,001, ^cdf=4, F=25,051 p<0,001. HCC= hair cortisol concentrations, PSS = perceived stress scale

Associations between HCC and PSS

There was a very weak negative correlation between the logarithmic values of HCC and PSS scores in the whole study population of both IBS- and non-IBS patients. When considering the large sample size such a weak correlation is probably not meaningful (Figure 7). Also, no significant correlation was seen when the two groups were analyzed separately, (IBS: $Rho=-0,08$, $p=0,32$, non-IBS: $Rho=-0,11$, $p=0,07$).

Figure 7. Scatterplots of the relationship between logarithmic HCC and the total scores of PSS in the whole TWIBS population. Spearman's $Rho=-0.129$, $p=0.005$. HCC= hair cortisol concentration, PSS= perceived stress scale, TWIBS= the twin cities IBS study.

Paper II

Group comparisons of self-rated health and PHC utilization

As seen in Table 5, a smaller amount of IBS patients reported good health, and IBS patients also had substantially more primary health care contacts than the control group of primary care patients without GI complaints.

Predictors of good self-rated health

Univariate and multivariate logistic regression analyses of the probability of IBS and non-IBS patients to report good health are reported in Tables 9-10. Only younger age, fewer GI symptoms and higher SOC were found to be statistically significant, independent predictors of good self-rated health in the multivariable analyses as well as the last step of the backward reduction model in both IBS and non IBS-patients (Tables 9-10), although other factors were associated univariately.

Table 9. Univariate and multivariable logistic regressions for the odds of IBS patients reporting good health

	n	OR	Univariate		Full multivariable model			Reduced multivariable model		
			95% CI	p	OR	95% CI	p	OR	95% CI	p
Age	183	0.97	0.95-0.99	0.01	0.95	0.92-0.98	<0.01	0.95	0.92-0.97	0.01
Male	183	1.61	0.78-3.33	0.20	1.84	0.74-4.59	0.19			
Number of comorbidities	182	0.95	0.92-0.98	<0.01	0.98	0.93-1.03	0.46			
Depression	182	0.67	0.34-1.31	0.24	0.85	0.35-2.08	0.72			
Anxiety disease	182	0.66	0.34-1.21	0.18	1.11	0.47-2.65	0.81			
Sleep disorder	182	0.47	0.20-1.06	0.07	0.86	0.31-2.41	0.77			
Extraintestinal pain disorders	185	0.37	0.18-0.76	0.01	0.55	0.21-1.44	0.23			
Ave days of abd. pain /week	175	0.83	0.73-0.95	0.01	0.84	0.74-0.98	0.03	0.83	0.72-0.96	0.01
Ave hours of abd pain/ week	175	0.99	0.98-1.00	0.01	n/a	n/a	n/a			
Ave episodes of bloating/ week	175	0.88	0.77-0.99	0.05	n/a	n/a	n/a			
Perceived stress	178	0.97	0.93-1.01	0.10	1.03	0.97-1.10	0.35			
Sense of coherence	181	1.05	1.02-1.08	<0.01	1.08	1.03-1.13	<0.01	1.07	1.03-1.11	0.01

n/a=not applicable due to multicollinearity, Ave= average, abd= abdominal.

Table 10. Univariate and multivariable logistic regressions for the odds of non-IBS patients reporting good health

	n	Univariate			Full multivariable model			Reduced multivariable model		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Age	360	0.99	0.96-1.02	0.57	0.95	0.91-1.00	0.03	0.96	0.91-1.00	0.04
Male	360	1.98	0.87-4.51	0.10	1.95	0.64-5.91	0.24			
Number of comorbidities	346	0.90	0.85-0.94	<0.01	0.93	0.87-1.00	0.05			
Depression	346	0.30	0.12-0.78	0.01	0.47	0.13-1.75	0.26			
Anxiety disease	346	0.34	0.15-0.77	0.01	0.80	0.25-2.62	0.72			
Sleep disorder	346	0.68	0.19-2.41	0.55	5.54	0.81-37.97	0.08			
Extraintestinal pain disorders	346	0.26	0.10-0.65	<0.01	0.41	0.13-1.29	0.13			
Ave days of abdominal pain /week	353	0.63	0.47-0.84	<0.01	0.62	0.43-0.89	0.01	0.61	0.43-0.88	0.01
Ave hours of abdominal pain/ week	353	0.98	0.96-1.00	0.01	n/a					
Ave episodes of bloating/ week	353	0.67	0.54-0.83	<0.01	n/a					
Perceived stress	352	0.98	0.96-1.00	0.01	0.98	0.91-1.05	0.56			
Sense of coherence	358	1.07	1.03-1.10	<0.01	1.04	0.99-1.10	0.13	1.07	1.02-1.11	<0.01

n/a=not applicable due to multicollinearity, Ave= average, abd= abdominal.

Predictors of many PHC contacts

Univariate and multivariate logistic regression analysis of the probability of IBS and non-IBS patients to have many PHC contacts are presented in Tables 11-12. Only number of comorbidities and comorbid sleeping disorder remained significant, independent predictors of many PHC contacts in the multivariable analyses, as well as the last step of the backward reduction model of IBS patients (Table 11). In the final analyses of the control group of non-IBS patients, number of comorbidities and perceived stress remained significantly associated with many PHC contacts (Table 12). Other factors were also associated univariately in both groups.

Table 11. Univariate and multivariable logistic regressions for the odds of IBS patients having many PHC-contacts

	n	Univariate			Full multivariable model			Reduced multivariable model		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Age	185	1.01	0.99-1.04	0.31	0.99	0.95-1.04	0.79			
Male	185	2.29	0.83-6.29	0.11	1.11	0.26-4.85	0.89			
Number of comorbidities	185	1.23	1.15-1.32	<0.01	1.20	1.10-1.31	<0.01	1.23	1.14-1.32	<0.01
Depression	185	2.67	1.29-5.53	0.01	1.57	0.49-5.05	0.45			
Anxiety disorder	185	5.41	2.60-11.26	<0.01	2.50	0.71-8.86	0.16			
Sleep disorder	185	8.25	3.41-19.97	<0.01	3.82	1.22-11.97	0.02	5.76	2.00-16.57	<0.01
Extraintestinal pain disorders	185	25.90	3.46-193.61	0.02	5.43	0.60-48.73	0.13			
Ave days of abdominal pain /week	175	1.03	0.89-1.20	0.69	0.93	0.76-1.14	0.49			
Ave hours abdominal pain/week	175	1.00	0.99-1.01	0.65	n/a	n/a	n/a			
Ave episodes of bloating/ week	175	1.03	0.89-1.19	0.65	n/a	n/a	n/a			
Perceived stress	174	1.04	1.00-1.09	0.08	1.01	0.93-1.10	0.78			
Sense of coherence	180	0.97	0.94-0.99	0.02	0.99	0.93-1.06	0.76			

n/a=not applicable due to multicollinearity, Ave= average, abd= abdominal.

Table 12. Univariate and multivariable logistic regressions for the odds of non-IBS patients having many PHC-contacts

	n	Univariate			Full multivariable model			Reduced multivariable model		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Age	348	0.99	0.96-1.02	0.48	0.98	0.93-1.03	0.36			
Male	348	0.61	0.25-1.51	0.29	0.36	0.09-1.40	0.14			
Number of comorbidities	346	1.30	1.21-1.41	<0.01	1.31	1.18-1.45	<0.01	1.31	1.20-1.43	<0.01
Depression	346	5.04	2.08-12.18	<0.01	0.23	0.04-1.55	0.13			
Anxiety disorder	346	8.06	3.62-17.95	<0.01	4.16	1.18-14.63	0.03			
Sleep disorder	346	3.87	1.42-10.60	0.01	1.28	0.24-6.70	0.77			
Extraintestinal pain disorders	346	6.53	2.22-19.20	<0.01	2.55	0.58-11.16	0.21			
Ave days of abdominal pain /week	342	1.35	0.96-1.89	0.08	0.84	0.46-1.54	0.57			
Ave hours abdominal pain/week	342	1.02	1.00-1.04	0.10	n/a	n/a	n/a			
Ave episodes of bloating/ week	342	1.32	1.03-1.68	0.03	n/a	n/a	n/a			
Perceived stress	340	1.13	1.07-1.19	<0.01	1.09	0.99-1.20	0.10	1.12	1.04-1.19	<0.01
Sense of coherence	346	0.93	0.89-0.96	<0.01	0.98	0.92-1.05	0.57			

n/a=not applicable due to multicollinearity, Ave= average, abd= abdominal.

Area under the curve of the regression models

The area under the receiver operating characteristic (ROC) curves for the final models of the regression models for self-rated health and PHC utilization in both the IBS and the control group of non-IBS patients are presented in Figure 8 A-D, and indicate useful (0.74) to strong (0.93) discrimination.

Figure 8. Receiver operating characteristic (ROC) curves of the logistic regression models presented for the odds of: **A)** IBS patients reporting good health considering age, average days of abdominal pain per week and sense of coherence as independent factors, **B)** non-IBS patients reporting good health considering age, average days of abdominal pain per week and sense of coherence as independent factors, **C)** IBS patients having many PHC contacts considering age, gender, number of comorbidities and sleep disorder as independent factors. **D)** non-IBS patients having many PHC contacts considering number of comorbidities and perceived stress as independent factors. AUC= area under the curve, HCU= health care utilization, SRH= self-rated health.

Paper III

Group comparisons of TNF- α and fatigue impact

Fatigue impact was nearly fourfold higher in IBS patients (median=43.0 (IQR=32)) compared to HC (median=11.0 (IQR=18); $p < 0.001$).

Further, IBS patients demonstrated significantly higher plasma levels of TNF- α (median=5.1, IQR=1.5) than HC (median=4.5, IQR=1.7) (Figure 9).

Figure 9. Plasma levels of TNF- α in IBS patients and HC, $p=0.001$.

Within group correlations

Plasma levels of TNF- α showed a positive correlation with fatigue impact in IBS patients, but not in HC (Figure 10). Further within group correlational findings between TNF- α as well as fatigue impact and relevant background variables as well as EALs are presented in Table 13. In summary, a slight negative association was seen between male sex and TNF- α . Both fatigue impact and TNF- α were related to IBS symptom severity in patients. With respect to psychological symptoms, fatigue impact was positively associated with anxiety as well as depression in both groups. Three out of 5 categories of childhood trauma were related to fatigue impact in IBS patients, whereas no other significant association in either IBS patients or HC were detected.

Table 13. Within group correlations to TNF- α and mFIS

	TNF- α		mFIS	
	IBS	HC	IBS	HC
Age	-0.01	-0.20	0.08	-0.13
Male sex	-0.20*	-0.03	-0.20	0.22
Body Mass Index	-0.02	0.09	0.05	-0.26
Psychotropic medication	0.16	-	0.17	-
HADS Anxiety	0.09	0.06	0.43**	0.67**
HADS Depression	0.17	-0.06	0.63**	0.42**
IBS-SSS	0.25*	-0.05	0.36**	0.26
CTQ Emotional abuse	-0.07	0.20	0.37**	0.10
CTQ Physical abuse	-0.14	0.24	0.13	-0.05
CTQ Sexual abuse	0.04	0.23	0.12	0.02
CTQ Emotional neglect	<0.01	0.27	0.23*	0.09
CTQ Physical neglect	<0.01	0.03	0.35**	0.02

Spearman's correlation coefficients indicating associations with fatigue impact and TNF- α of age, sex, BMI, psychotropic medication, psychological symptoms, and IBS symptom severity separately for IBS patients and HC. IBS = Irritable bowel syndrome, HC = healthy controls, mFIS = modified Fatigue Impact Scale, HADS = Hospital Anxiety and Depression Scale, IBS-SSS = IBS Symptom Severity Scale. CTQ= childhood trauma questionnaire. *Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

Figure 10. Scatterplots of the relationship between TNF- α levels and scores of the modified Fatigue Impact Scale (mFIS) in IBS patients (Spearman's Rho = 0.28, $p=0.001$) and healthy controls (HC) (Spearman's Rho = 0.13, $p=0.37$).

The positive association between TNF- α and fatigue impact in IBS patients (Figure 10) was controlled for gender, age, BMI, use of psychotropic medication, and HADS scores of depression, and anxiety using a multiple regression backward elimination procedure. Only fatigue impact was a statistically significant predictor ($\beta=0.26$, $B=0.02$, $SE=0.01$, $p=0.009$) in the final model ($R^2=0.07$, $F=5.90$, $p=0.02$).

Associations to childhood trauma

As seen in Table 13, there were positive correlations between mFIS and emotional abuse as well as physical and emotional neglect in IBS patients. No associations between childhood trauma and fatigue was seen in the control group and no associations between childhood trauma and TNF- α was seen in either group. When controlling for age, sex, and HADS only emotional abuse remained associated to fatigue impact in IBS patients (Table 14).

Table 14. Multiple regressions of mFIS and CTQ variables in the IBS group

	1. mFIS v Emotional abuse (β)	2. mFIS v Emotional neglect (β)	3. mFIS v Physical neglect (β)
1. Emotional abuse	0.20*		
2. Emotional neglect		0.06	
3. Physical neglect			0.18
Male sex	-0.17	-0.20**	-0.21*
Age	-0.03*	-0.02	-0.04
HAD anxiety	0.02	0.03	0.02
HAD depression	0.49**	0.56**	0.52**
	R ² =0.43, F=11.55	R ² =0.40, F=10.36	R ² =0.43, F=11.49

Associations between mFIS and the three CTQ variables with significant correlations, in three separate models controlling for sex, age and psychiatric comorbidity. Unstandardized coefficients are presented. mFIS= modified fatigue impact scale, HADS = Hospital Anxiety and Depression Scale, CTQ= childhood trauma questionnaire. β = the standardized regression coefficient. *Association is significant at the 0.05 level (2-tailed). ** Association is significant at the 0.01 level (2-tailed).

RsfMRI analyses

As expected, a functionally connected network of the 7 selected mesocorticolimbic regions, which have previously been related to fatigue, were confirmed in all participants. Figure 11 displays the connectome ring and how the network ROIs are functionally connected to each other. The NAc, particularly exhibited functional connectivity to all other ROIs. Only when taking fatigue impact into consideration, there was a difference in ROI-to-ROI functional connectivity between IBS and HC ($p_{\text{FDR}} > 0.05$). These associations will be discussed further below. There were no significant associations between functional connectivity in the selected nodes and plasma levels of TNF- α in either IBS or HC.

Figure 11. Significant functional connectivity between regions of interest comprising the mesocorticolimbic network. Data are presented as a connectome ring with false discovery rate correction ($p_{\text{FDR}} < 0.05$). ACC = anterior cingulate cortex; amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; ROI = region of interest; L = left; R = right. The color bar represents T-values for the ROI-to-ROI effects.

In individuals with IBS, fatigue impact negatively correlated with connectivity between the NAc and the DLPFC in both hemispheres (Figure 12A), whereas in HC, fatigue impact positively correlated with connectivity between the right NAc and bilateral amINS (Figure 12B).

The between-group analysis showed that IBS patients had less functional connectivity between the right NAc and bilateral amINS than HC in relation to fatigue impact (Table 15). It is important to note that the observed significant difference between groups may be driven by *positive associations* between fatigue impact and the functional connectivity in HC in contrast to the *absence of such association* or trend thereof in IBS.

Between-group analysis further revealed IBS patients to exhibit lower fatigue impact-related connectivity between the right NAc and the right DLPFC, but stronger inter-insular connectivity between hemispheres compared with HC (Table 15).

There were no significant associations between functional connectivity in the selected nodes representing the mesocorticolimbic network and plasma levels of TNF- α in either IBS or HC (data not shown).

Figure 12. Associations between fatigue impact and functional connectivity in IBS (A) and in healthy controls (HC) (B). Blue signifies negative correlations between fatigue impact and connectivity, and red signifies positive correlations. amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right.

Table 15. Significant relations between mFIS and functional connectivity.

Group	Regions	t-value	p _{FDR}
IBS			
	L NAc – L DLPFC	-3.05	0.019
	R NAc – R DLPFC	-2.81	0.038
HC			
	R NAc – L amINS	3.38	0.009
	R NAc – R amINS	3.09	0.011
IBS > HC			
	R NAc – L amINS	-2.28	0.049
	R NAc – R amINS	-2.46	0.049
	R NAc – R DLPFC	-2.28	0.049
	R amINS – L amINS	2.52	0.045

Significant results from the region of interest analyses of the relation between fatigue impact and connectivity in the selected network. Results are controlled for anxiety and depression and corrected for multiple comparisons. amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right; HC = healthy controls; p_{FDR} = false discovery rate corrected p-value.

DISCUSSION

The aim of this thesis was to explore aspects of the biopsychosocial model in IBS related to stress, comorbidities, and fatigue. Since study I and II are performed in a primary care setting, it also takes on that perspective. Figure 13 illustrates where the main results of this thesis fit into the biopsychosocial model. As far as we know, all the main findings either add some new knowledge to the vast bulk of IBS research, or add weight towards a certain direction where previous literature is divergent. New insights in this thesis as well as the relation to previous knowledge will be discussed in the respective sections *stress*, *comorbidities* and *fatigue* below. In both study populations of this thesis the background material also shows many health disadvantages in the IBS group. The socioeconomic status was lower in the IBS group of both studies and particularly in the TWIBS study the previous known burden of comorbidities in IBS is also made elusive in the background tables. IBS patients also had more self-perceived stress, lower self-rated health and more health care consumptions, as discussed further below.

Figure 13. IBS-Biopsychosocial Conceptual model of pathogenesis and clinical expression showing only factors investigated in this thesis as well as main results. HPA axis= hypothalamic- pituitary- adrenal axis, ENS= the enteric nervous system, CNS= the central nervous system. HCC = hair cortisol concentration, TNF-α = tumor necrosis factor-α, EAL = early adverse life events.

Stress

To compare levels of cortisol in hair concentration as well as perceived stress between IBS patients and other primary care patients.

To investigate if biological stress as measured with HCC correlates with perceived stress in IBS and non-IBS patients.

Both perceived stress and HPA axis activity is associated with IBS in this thesis as well as in previous literature (37, 53, 61). The most interesting finding in paper I of this thesis was low HCC in a considerable portion of IBS patients despite significantly higher perceived stress in the IBS group compared to other PHC patients. This adds evidence for a possible suppressed HPA axis in a subgroup of IBS patients.

Our results suggest that HCC can be a meaningful and non-invasive measure of HPA axis activity over time, to use in IBS research.

It is previously known that the HPA axis, as well as the ANS are involved in the development of disorders of gut-brain interaction and the perpetuation of IBS symptoms (23, 35, 36). It is also very likely that the HPA axis activity is altered in IBS. Previous research is however divergent. Some have suggested a subdued cortisol production in response to stress (62) or a dexamethasone test (65) in IBS and that is in line with our results showing relatively low HCC levels in a substantial portion of IBS patients.

There are many possible explanations for this suggested hypocortisolism. The burden of a chronic disease and the stress response related to GI symptoms could in the long run hypothetically cause a suppressed or dysregulated HPA axis.

Another reason could go back to adverse events and environment of childhood. Results suggesting a suppressed HPA-axis have previously been demonstrated in both post-traumatic stress syndrome (213) and in patients with a history of childhood trauma (214). It is known that childhood trauma is associated with IBS (215) and it has also been demonstrated that early adverse life events could be associated with altered HPA axis activity in IBS (216). Unfortunately, we do not know if the subset of our patients with low HCC also had a history of previous trauma. No association was however seen between HCC and reported stressful events during the last year.

Alongside having an IBS diagnosis, female gender, living in a blue-collar town, and psychiatric comorbidity were as IBS, albeit weak, also independently, negatively associated with quintiles of HCC. The sex difference in levels of HCC is previously known and probably physiological (74). Living in a blue-collar town could maybe be associated with chronic stress, which according to the meta-analysis of Stalder first leads to elevation but as time goes a decline of HCC (83). Regarding the higher HCC also in

smokers, that is in line with previous research reporting an altered HPA-axis also as a consequence of smoking (226), but associations have previously not been established with HCC (83).

In the TWIBS population of IBS-patients, we found no association between perceived stress i.e. the cognitive appraisal of stress and HCC, as a putative measure of a physiologic response to both exteroceptive and interoceptive stress exposure. This finding is in analogy with the meta-analysis of stress-related and basic determinants of HCC, that was conducted at the same time as the analyzes of this study (74). Both stress measures were however independently associated with IBS. In the whole population of both IBS and non-IBS patients, a weak negative association between the two stress measures was seen, which possibly could be an artefact of the very large sample size available yielding high statistical power for a weak correlation. A disconnection between subjective distress and cortisol secretion in experimental test-situations is also previously reported. Other factors such as recognizability and controllability of the stressor seems to be of more importance (217-222).

Comorbidities

To evaluate the unique associations of comorbidities, GI symptoms, perceived stress, and sense of coherence with health care utilization and self-rated health in IBS patients and a control group of other patients.

In paper II we demonstrated that approximately 40 percent of the IBS patients rated their health as poor while only about 10 percent of the non-IBS patients did, and that IBS patients also had more than double the number of PHC contacts on average, in comparison to the control group.

Self-rated health has previously been shown to be a strong, independent predictor of both morbidity and mortality (223). Our finding of lower self-rated health in IBS patients compared with other primary care patients, thus highlights that IBS patients constitute a vulnerable group in the need of special attention.

Understanding common underlying causes for the many health care contacts of IBS patients is also valuable for proper use of clinical resources. The associations of comorbidities as well as GI symptoms, and psychological factors with self-rated health and health care utilization were investigated in this thesis.

One quite surprising, and interesting finding was that IBS patients with comorbid sleep disorders had over five times the odds of having many PHC contacts, compared to those without a diagnosis of sleep disturbances.

Could that association simply mirror possible frequent contacts for renewal of sleep medication prescriptions? The association between sleep disorders and health care contacts was however not found in the control group of primary care patients without GI complaints, which suggests another specific link between sleep disturbances and health care utilization of IBS patients. Sleep disturbances have previously been associated with GI disease in general and with IBS in particular (224, 225). Poor sleep can exacerbate GI symptoms (226), and conversely, many GI diseases affect the sleep-wake cycle, leading to poor sleep (225). A randomised, controlled trial evaluating the effect of melatonin in patients with IBS showed significantly reduced GI symptoms, but no difference in polysomnography between the patients treated with melatonin and those treated with a placebo (227).

When planning study II of this thesis, according to previous literature (116), we had hypothesized that certain particular comorbidities such as extraintestinal pain syndromes and psychiatric diagnoses, alongside perceived stress and SOC would be more important for both the self-rated health and the PHC utilization of IBS patients, then number of comorbidities and GI symptoms. Comorbidities however (and sleep disorders in particular) turned out, only to be related to PHC utilization but not to the self-rated health of IBS patients. We investigated both total numbers of comorbidities, as well as some comorbidities of particular interest, namely depression, anxiety disease, sleep disorders and extraintestinal pain disorders. Good self-rated health was found uniquely associated with younger age, fewer days of GI pain per week, and higher SOC in both IBS and non-IBS patients.

It has previously been demonstrated that pain, fatigue, and low mood drive poor self-rated health, which further could be due to an association with low-grade inflammation (228-230). The association between inflammation and self-rated health was not part of the research questions of this thesis, but the findings still partly supports that idea, with the identified unique associations between self-rated health and abdominal pain, as well as age in both groups. Pain is considered part of the sickness behavior associated with inflammation and levels of blood inflammatory markers are also known to rise with ageing (231).

In both IBS- and non-IBS patients also higher SOC was associated with better self-rated health. It seems that part from individualized treatment of abdominal pain, help to strengthen the patients' SOC could be one way of achieving better self-rated health, not only in IBS-, but in all PHC patients. Even though sense of coherence previously has been considered a stable entity that is developed in young adulthood, some more recent studies report on successful interventions in strengthening the SOC in adult populations (94, 232-235).

Fatigue

To investigate associations between fatigue and levels of a pro-inflammatory cytokine (TNF- α) as well as a possible role of a resting state network of mesocorticolimbic regions known to be related to fatigue.

Additionally: to investigate if there is an association between EAL and fatigue impact of daily lives as well as TNF- α in IBS.

As has been found in previous research, IBS patients were substantially (fourfold in our data) more affected by fatigue compared with HC in study III of this thesis (144, 236). The higher levels of TNF- α in IBS patients compared with HC also supported previous research suggesting associations between immune activation and IBS (120, 122, 123, 142).

Further, we found a positive association between fatigue impact and TNF- α , which complements existing scarce and inconsistent knowledge. For example, in one study of female IBS patients, elevated TNF- α was associated with comorbid chronic fatigue syndrome (142), while another group found no association between fatigue impact and levels of proinflammatory cytokines including TNF- α (141).

Our results add evidence that fatigue in IBS could, be part of a sickness response or a, so called, sickness behavior due to a dysregulated immune system, as has been suggested for many other chronic conditions (165). We found a unique association between TNF- α and fatigue impact in IBS, independent of possible confounders such as sex, age, BMI, medication and even depression and anxiety.

Additionally, for this thesis frame, associations between early adverse life events and fatigue impact as well as TNF- α were also analyzed. No associations between the specified kinds of childhood trauma and TNF- α were found. Fatigue impact, on the other hand, was positively associated to childhood traumas of emotional abuse as well as emotional and physical neglect. When controlling for HADS, only emotional abuse remained associated to fatigue impact, suggesting that in patients with early adverse life events, experiences of fatigue could be due to comorbid psychiatric disorders and driven by other factors than TNF- α , albeit other proinflammatory cytokines not excluded. Also in this system, there is a known cross-talk between the immune system and the HPA axis (237). Increased activity of the HPA axis has an inhibitory effect on the production of pro-inflammatory cytokines and vice versa (238). The cytokines TNF- α , IL-1 β and IL-6 have previously been associated with release of CRF and HPA axis activation (51, 239). Measures of the HPA axis was however not studied in the Brain-Gut study, which the study III of this thesis, was based on.

We further investigated a network of regions known to be related to fatigue. As far as we know, central correlates of fatigue have not previously

been studied in IBS. Using a hypothesis-driven approach, we were able to confirm a functionally connected network of mesocorticolimbic regions of known associations to different dimensions of fatigue in both IBS patients and HC.

The NAc, the main hub in the mesolimbic dopamine circuit involved in the cognitive processing of motivation aversion and reward (164), seemed to have a central role in the network since, unlike the other regions, it was found to be functionally connected to all the others.

When taking fatigue impact into consideration, IBS patients had a general tendency toward reduced functional connectivity within this network in comparison to controls. In IBS, fatigue impact was negatively correlated with the connectivity between NAc and DLPFC bilaterally, while in controls it was positively correlated to the connectivity between the right NAc and amINS in both hemispheres.

Our results are in line with some previous research in other patient groups with fatigue and other overlapping symptoms. Specifically, reduced functional connectivity has previously also been demonstrated in chronic fatigue syndrome (240), post traumatic stress syndrome (241) and depression (242). In a study of MS-related fatigue, reduced functional connectivity of the NAc and sensorimotor as well as reward networks was also observed (177).

We could not find evidence supporting an association between the functionally connected mesocorticolimbic network and levels of the proinflammatory cytokine TNF- α , even though extensive previous research has indicated an association between inflammation and central alterations in fatigue (151, 165). One reason for the contrary results of this study could be due to limitations of just measuring one cytokine. A previous study reported an association between increased plasma IL-6, IL-1 β and IL-1 receptor antagonists with decreased functional connectivity within a corticostriatal reward circuitry in depression (242). TNF- α was also measured in that study, but like in our study, no association with connectivity of striatal ROIs was found.

Unfortunately, despite measuring mFIS, sleep disturbances were not specifically assessed for in the Brain-Gut study. In multiple sclerosis, sleep disorders are strongly linked to fatigue and the treatment of sleep disorders was able to improve symptoms there of (243). Disruptions in functional connectivity of the insular cortices have also been reported to correlate with several sleep-related, cognitive, and psychological parameters (244). We also know from the TWIBS population as well as from previous research, that sleep complaints are common in IBS (224, 225) and that sleep disturbances are associated with immune activation (245). However, previous research has also established that actual objective sleep alterations are not

present in IBS (246), leading to the question of whether sleep complaints in this matter actually could be consistent with the experience of fatigue as part of the sickness response to inflammation, previously described.

Strengths, limitations, and reflections regarding the scientific process

A major strength of this thesis is that it is based upon two robust and relatively large, well-defined study populations. Another strength is the multimodal approach of study III (based on the Brain-Gut study). Both the TWIBS- and the Brain-Gut study, however, take on a cross-sectional design, which in its nature has the major limitation of not permitting any conclusions of causality even though it can give some guidance.

Time discrepancy

One recurrent limitation of this thesis is the time discrepancy between different measures. In the TWIBS study HCC is a measure of the past three months, PSS asks for a summary of the previous month, SOC only asks for how the patient usually feel and self-rated health is a question about how the participants rate their health in the present moment. Data about PHC utilization, as well as comorbidities, were collected for the whole 5-year period of the study. This could maybe explain why we do not see some associations that would have been expected. In the Brain-Gut study TNF- α , behavioral scales, and connectivity analyses were gathered and conducted at different time points and times of the day, which could have theoretical influence on the results. However, no systematic differences emerged between IBS and HC.

Weak associations

Further, a general limitation to this thesis, is that a number of the associations demonstrated for paper I and II are rather weak, indicating that many factors each very weakly affect the outcome. The results of those studies should be considered in the light of that, only seen as guidance and not absolute truths.

Health care registry

Data regarding diagnosis and health care utilization were gathered from the health care registry, which is a gold mine for the researcher in the county of Östergötland and a strength in getting complete data. However,

a general limitation in that case could be the influence of health economic matters such as health care funding and government policies when it comes to registering diagnoses. Also, the availability of healthcare has an obvious influence on the individuals' health care utilization.

Missing information

In both the TWIBS- and the Brain-Gut study numerous variables and relevant confounders, are evaluated. However, in terms of HPA axis and inflammation there are some variables missing, that would have been of value.

When investigating immune activation, clearly more cytokines or other immune markers, than just TNF- α would have added more information to the research questions of study III.

For paper I, we do not have data on either pregnancy, alcohol consumption or BMI, which are all factors that could influence cortisol levels. For paper III, comorbid sleep disturbances would have been of value to control for but was not asked about.

Research questions

Some of the research questions for this thesis and the decisions for data analyzes have been developed along my PhD education, while digging deeper into the vast bulk of previous research of IBS, comorbidities, and fatigue. To in some ways formulate new research questions along the way, out of questions that emerge due to more knowledge and insight is common, but not always optimal. Despite the clear disadvantages to the study design, it is however from an ethical point of view a way, of proving respect to the individuals that have volunteered, in making the most of the gathered information.

Errors in published material

While writing this thesis frame, some errors of published material unfortunately were discovered. Three numbers in the inclusion process of non-IBS patients in paper I, were wrongly calculated (Figure 1 of paper I). This mistake most likely happened since participants could be excluded for several different reasons. The numbers of non-IBS patients that agreed to participate should be 466 and those excluded due to steroid treatment should be 31. In total 316 non-IBS patients were left for final analyses in paper I. This is presented correctly in Figure 3 above. Further, for Table 1 of paper I, the ages of IBS and non-IBS patients were unfortunately mixed up. IBS patients were reported older than non-IBS patients, but really it was the other way round. This was due to a typing error and all other analyses including

age were made with the correct numbers. The ages of IBS- and non-IBS patients for study I are presented correctly in Table 3 above. Further the correlation regarding smoking is reversed in Table 3 of paper I. There is a slight negative correlation between non-smokers and HCC and not the other way around. All the other correlations are correctly interpreted in paper I, but the variables in Table 8 for the thesis frame are re-named for clarity.

I deeply regret these errors, but fortunately they do not change the main results of this thesis or paper I. However some precaution could be taken regarding the age differences between IBS and non-IBS patients since HCC is known to increase with age (247). Age was however controlled for in the multiple regression analyses, and IBS remained associated to quintiles of HCC nevertheless, while age did not. All together from the PhD student's perspective these errors have taught me much about how to practically handle data and what precautions to take in the processes of future research conduction, analyzes and presentation.

Several concepts and phenomena

As this thesis has evolved, I have come across several concepts and phenomena that each, and everyone for itself could have served as a ground for a thesis. In touching so many different and complex areas this thesis could be criticized for being too shallow, and only scratching on the surface. I have however tried to put these different aspects together in the biopsychosocial model of IBS. Keeping all different aspects in mind at the same time is also the art of general medicine, where I do my clinical work, and where most IBS patients consult.

CONCLUSIONS

Brain-gut interactions including stress, as well as sickness behavior as a putative response to immune activation in the light of a biopsychosocial model, also considering the wear and tear of everyday life stress, comorbidities and health outcomes, are investigated in this thesis. Although this work was not able to answer all its questions definitively, this thesis strengthens some previous ideas of mechanisms and points into directions of where to go further.

Firstly, the vulnerability of IBS patients is highlighted, both regarding low self-rated health, a strong predictor of future morbidity and mortality alongside an already established high healthcare consumption, as well as regarding poor socioeconomic status and burden of comorbidities. All in all, IBS patients consists a group of patients, the general practitioners need to understand and provide proper care for.

Regarding pathophysiological mechanisms this thesis adds evidence for a possible suppression of HPA axis activity in a substantial portion of IBS patients. Along with previous research, in other conditions, it also supports a disconnection between perceived stress and HPA axis activity in IBS and other primary care patients. That could be either due to the method of measuring perceived stress or else adds evidence that temporal everyday stress, at least to certain degrees, does not influence HPA axis activity.

Further comorbidity in general, and particularly sleep disturbances seem to be important for the PHC utilization of IBS patients, but comorbidities did not influence the self-rated health in neither IBS- nor non-IBS patients. Proper treatment of sleep disorders in IBS patients could possibly help minimizing excess health care contacts. Moreover, alongside treating GI pain, efforts to improve the individuals' SOC could be one way to achieve better self-rated health in both IBS and non-IBS patients.

Finally, this thesis also gives first evidence that the extraintestinal symptom of fatigue in IBS patients is associated with alterations in the connectivity within a mesocorticolimbic network where the NAc and the motivational aspect of fatigue seem to have a prominent role. We further add some evidence that fatigue in IBS could be associated with immune activation and part of a, so so-called, sickness behavior as well as having an association with previous childhood trauma. All together the findings of this thesis give rise to a broad variety of new minor and major research questions that will be further discussed below.

Future directions and clinical applications

Even though much research has been done, and the map of the biopsychosocial model in IBS is slowly becoming clearer, there is still much that is unexplored, accompanied by an urgent and unmet need for efficient treatment.

Studying mechanisms through interventional studies

To further understand mechanisms in IBS regarding both HPA axis, immune activation, and brain alterations, as well as associations to both GI symptoms and the burden of extraintestinal symptoms, such as fatigue, longitudinal studies are warranted. However, if pathophysiologic mechanisms possibly go as far back as to childhood adversities such a longitudinal study is hardly feasible. Instead interventional studies also considering mechanisms such as HPA axis activity, immune functioning and brain functioning would both shed some light on the complex pathophysiology of IBS, and at the same time hopefully improve treatment.

HPA axis activity, immune function, and childhood trauma

Regarding specific pathophysiological mechanisms, this thesis raises questions about the influence of childhood trauma on both HPA axis activity as well as immune function. A follow up study of HCC in the TWIBS population, also asking the participants regarding early adverse life events, could give some answer as to whether the putative suppression of the HPA axis, found in paper I, had an association with childhood trauma. At the same time, such a follow-up study, would add information about HCC level variations over time. Including other measures such as CRF levels, and diurnal changes of cortisol would also give further information about the possible altered HPA axis in IBS patients. Measures of immune activation such as TNF- α and other cytokines could also add further information about the cross-talk between the HPA axis and the immune system.

Likewise, a follow up study of study III, including HPA axis activity, would add information about pathophysiologic mechanisms of fatigue, and the putative association to also neuroendocrine alterations. Regarding fatigue, also a more extensive investigation of immune activation, both regarding peripherally and possibly also with measurements of central immune activation would be of interest.

Future studies regarding putative gender differences in IBS patients targeting both mesocorticolimbic connectivity, the experience of fatigue, and the relation to peripheral inflammation are also warranted.

Primary care considerations

As to suggestions from this thesis and of help to the primary care population of IBS patients, studies particularly targeting or considering comorbid sleep disorders as well as SOC and motivation are warranted. Both self-rated health and health care utilization are of interest to evaluate in treatment studies.

Considering the high PHC utilization of IBS patients with comorbid sleep disorder, also a follow-up study, looking closer into why IBS patients seek health care for sleep disturbances, would shed some light on this issue. In this matter, since some previous studies could not find actual objective sleep disturbances in IBS patients (246), a question is risen whether the perceived lack of sleep actually could mirror the extraintestinal symptom of fatigue, or be part of the same phenomenon of sickness behavior.

Finally, from the primary care perspective, it would also be of value to investigate whether the putative pathophysiological changes as well as possible improvement with an interventional study, as described above, would be specific to IBS, or be similar in other disorders associated with stress, possible immune activation and HPA axis alterations such as chronic pain, chronic fatigue syndrome or bodily distress syndrome, all also best understood with the biopsychosocial model in mind.

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