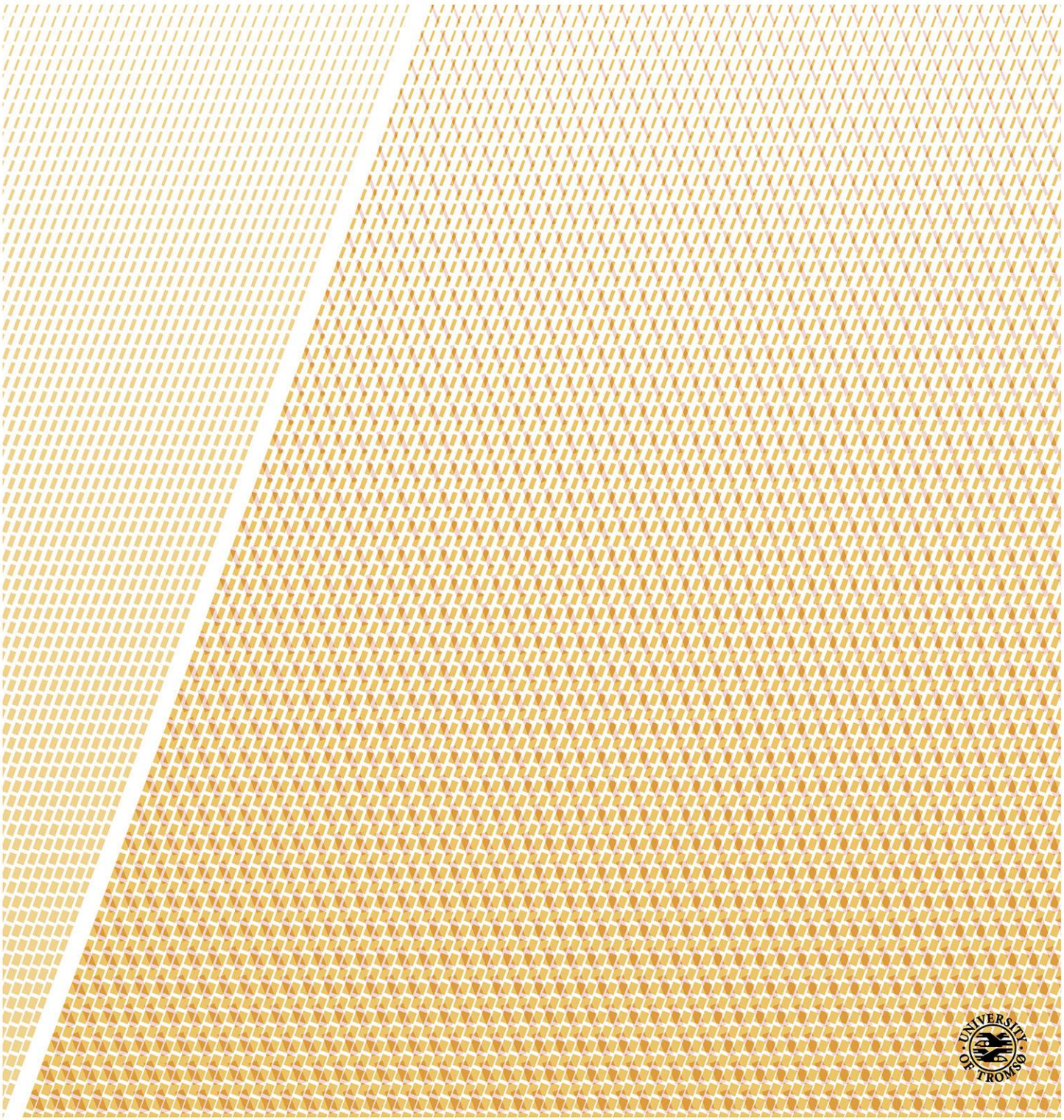
UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty of Health Sciences, Pediatric Research Group

Associations of widespread pain sensitivity, comorbid chronic pain and psychological distress with Irritable Bowel Syndrome and abdominal pain

Niklas Stabell

A dissertation for the degree of Philosophiae Doctor – June 2014



Contents

Summary

Background

Abdominal pain (AP) is a common symptom among children and adults. Irritable Bowel Syndrome (IBS) is reported to be one of the most frequent types of AP. Comorbid chronic pain and mental health problems are common in AP and IBS. There is increasing knowledge of a variety of biopsychosocial mechanisms contributing to AP and IBS. Among these, increased visceral and widespread pain sensitivity are factors possibly contributing to triggering and maintaining pain symptoms. However, it is not clear to what degree the association between widespread hyperalgesia and IBS is related to confounding with comorbid chronic pain and mental health. All previous IBS pain sensitivity studies have been limited to clinical samples, a majority only including premenopausal female adult patients, and there are no studies of community samples, including both sexes and pediatric populations. The main aim of this thesis was therefore to analyze the association of IBS and widespread pain sensitivity among adolescents and adults in the general population, controlling for sex, age, comorbid chronic pain and psychological distress. The second aim was to describe the association between specific abdominal pain dimensions, bowel symptoms and other chronic pain with depression among adolescents with chronic abdominal pain and IBS.

Methods

More than ten thousand adults (n = 10 566, age 30-87) and almost one thousand adolescents (n = 961, age 15-17) completed questionnaires and pain sensitivity assessment (heat, pressure and cold-pressor pain) in two cross sectional population based studies (2007/08 and 2010/11 respectively). Associations between self-reported symptoms of IBS and measured pain sensitivity were studied, controlling for sex, age, comorbid chronic pain and psychological distress (the Hopkins Symptom Check List). Further, associations between IBS, AP and different AP symptom dimensions with depression (the Short Mood and Feelings questionnaire) among the adolescent participants were studied in a multivariable regression model.

Results

The adult and adolescent IBS prevalence was 5.3 % and 8.2 % respectively (Rome II and III criteria respectively).

Among the adolescents pain thresholds were significantly lower in IBS cases compared to controls. The differences remained significant after adjusting for sex and comorbid pain (mean pressure difference fingernail = - 62 kPa with 95 % CI = -109 to -15, shoulder = - 46 kPa with 95 % CI = - 78 to – 13, mean heat difference = - 0.8 ^{o} C with 95 % CI = - 1.6 to - 0.04 and mean sum z-score difference = - 0.4 with 95 % CI = - 0.6 to - 0.17) , whereas only heat pain and mean sum z-score threshold difference were significant when additional adjust for mental distress was performed.

Similar findings for heat pain threshold were made for adults, where differences remained significant after adjusting for sex, age, comorbid chronic pain and psychological distress (mean difference: - 0.5 ^{o} C with 95 % CI = - 0.8 to - 0.1). However no difference in pressure pain supra-threshold was found after adjustments for sex and comorbid pain. Increased pain intensity (mean numeric rating score, 0 to 10, of 5.9 in IBS vs. 5.3 in controls, $p < .01$) and lower pain tolerance was found in the cold-pressor test (Hazard ratio = 1.3, with 95 % CI = 1.1 to 1.5) among adult IBS cases after the same adjustments, while no pain tolerance differences between adolescent IBS cases and controls were found. Both adolescent and adult participants with IBS and severe abdominal pain had the lowest heat pain thresholds. Among adults, these participants also had the highest pain ratings and lowest pain tolerance in the cold-pressor test.

Increased symptoms of depression were found among adolescents with both monthly abdominal pain (20.5 %) and IBS (24.7 %) compared to controls (8.1 %, both $p < 0.01$). In the multiple logistic regression analyses AP pain intensity and widespread pain distribution were significantly associated with depression (severe vs. mild pain: Odds ratio = 4.0, with 95 % CI = 1.5 to 10.7, multiple vs. single site AP: Odds ratio = 5.5 with 95 % CI = 2.6 to 11.8 and comorbid non-AP: Odds ratio = 3.3 with 95 % CI = 1.6 to 6.8 respectively). In contrast, sex and other abdominal symptoms, including those symptoms that distinguish IBS from other types of AP, were not significantly associated with depression.

Conclusion

This is the first report documenting widespread hyperalgesia among adolescent and adult individuals with symptoms of IBS in the general population. Results were found to be independent of sex, comorbid pain and psychological distress. Increased pain sensitivity may contribute in triggering and maintaining chronic pain, but prospective studies are needed to examine these possible causal relationships.

The prevalence of depression is considerably increased among adolescents with AP and IBS in the general population, in particular among adolescents with AP reporting severe and widespread abdominal pain, and among adolescents reporting comorbid chronic pain in other body sites. Evaluating these pain symptom dimensions may be of value for identifying subgroups adolescents with AP and IBS that have greater risk of depression.

List of papers

Paper 1

Increased pain sensitivity among adults reporting irritable bowel syndrome symptoms in a large population-based study. PAIN 154 (2013); 385–392

Niklas Stabell, Audun Stubhaug, Trond Flægstad, Christopher S Nielsen

Paper 2

Widespread hyperalgesia in adolescents with symptoms of Irritable Bowel Syndrome: Results from a large population-based study. Accepted for publication in the Journal of Pain (May 2014).

Niklas Stabell, Audun Stubhaug, Trond Flægstad, Emeran Mayer, Bruce Naliboff, Christopher S Nielsen

Paper 3

Associations between abdominal pain symptom dimensions and depression among adolescents. Accepted for publication in the Scandinavian Journal of Pain (April 2014).

Niklas Stabell, Trond Flægstad, Audun Stubhaug, Christopher S Nielsen

1. INTRODUCTION

1.1. Functional abdominal pain and Irritable Bowel Syndrome

The International Association for the Study of Pain (IASP) defines pain as: "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[1] Pain is always subjective, an individual unpleasant sensation, and therefore also an emotional experience. The unpleasant sensation is defined as pain irrespectively of cause and also if identifiable tissue damage or potential tissue damage is absent. Acute pain is one of our most important sensations to reduce and avoid tissue damage. In the medical literature, the definition of chronic pain in general and of specific chronic pain conditions is less consistent, regarding both the duration and frequency of the pain symptoms. [2,3] Acute pain is often related to peripheral inflammation or tissue damage (nociceptive input), whereas these peripheral factors are often less prominent or non-identifiable in many chronic pain patients.[4]

Functional pain and functional pain syndromes are characterized by chronic pain without identifiable structural, inflammatory, or biochemical abnormalities that could fully explain the symptoms. Functional abdominal pain and Irritable Bowel Syndrome (IBS) are in the same way basically defined by the patients' symptoms and are referred as disorders rather than diseases with an organic cause.[5]

Diagnosing functional abdominal pain and IBS remains a challenge with the absence of physical or anatomical markers. For more effective diagnosis of the different functional gastrointestinal disorders (FGIDs) a number symptom-based diagnostic criteria have been developed over the last 25 years and revised several times since the first Manning criteria for IBS of 1978.[6] In 1958 Apley and Naish defined recurrent abdominal pain (RAP) in a child as "at least three bouts of pain, severe enough to affect the child's activities, during at least a three-month period with attacks continuing the preceding year".[7] This RAP definition is widely adopted with many reports of the condition in the medical literature,[8] but in recent years it has been increasingly replaced by the classification system for FGIDs. In contrast to the FGID criteria[8], the RAP definition does not exclude organic diseases, and RAP is therefore a broader term and not exclusively a functional pain disorder.

One of the important roles of the classification system for FGIDs is to improve clinical diagnosis, distinguishing among different FGIDs from each other and from possible organic

diseases.[9] An additional aim of more accurate diagnostic, symptom-based criteria has been to improve the management and treatment of the FGIDs, in contrast to handling FGID as an exclusion diagnosis without available specific treatment or treating it as a psychosomatic disorder of mainly psychiatric origin.

Better classification systems have also led to increased research in the FGID field, with increased knowledge of the prevalence, comorbidity, and psychosocial consequences of the disorders.[10,11] Furthermore, increased attention to, and interest in the FGIDs have led to improved evidence of possible pathophysiological mechanisms,[5,12-16] which has also led to discussion on the origin of the disorders and if they actually are functional or organic by nature.[9]

The number of FGIDs has increased as the classification criteria have been revised; the latest criteria— the Rome III criteria of 2006—include 28 abdominal pain and bowel disorders for adults and 17 for children.[5] The system is organized by the anatomical localization of the symptoms (e.g., esophageal, stomach-duodenum, bowel, and anorectal symptoms). In the pediatric Rome criteria there are, in addition, age-specific FGID disorders (infant/ toddler and child/adolescent).

IBS is one of the most common FGIDs and is characterized by abdominal pain or discomfort, associated with altered bowel movements.[9,17] The symptoms are not very specific and mimic several organic gastroenterological diseases; IBS is for the same reason a clinical exclusion diagnosis. Still, the symptom-based IBS criteria perform fairly to moderately well compared to clinical IBS diagnostics, at least when alarm symptoms of organic diseases are included.[18] In one validation study of the different IBS criteria, the sensitivity ranged from 68.8% to 95.8% and specificity from 70.6% to 79.5% compared to clinical IBS diagnostics.[19] The Manning criteria included most clinical cases but were also the least specific. Few differences were found within the Rome criteria, but the Rome III criteria were found to be most specific. For children there are few similar validation studies of the Rome criteria for FGIDs, but most children referred to outpatient clinics with abdominal pain are reported to have a functional disorder according to the Rome criteria, with IBS as the most frequent.[20,21]

Most validation studies are done at specialized gastroenterologist clinics and few in primary care, where most patients with IBS are diagnosed and treated. The IBS Rome III criteria is in one study reported to identify three of four patients that had an IBS diagnosis by their general practitioner,[22] but others have reported poorer agreement between the IBS criteria

and clinical diagnoses in primary care.[23] To our knowledge, the question remains unanswered as to what degree IBS symptoms in the general population correspond to a clinical IBS diagnosis.

As mentioned, the diagnostic criteria of IBS have changed over time. The cardinal and associated symptoms are the same, but the criteria diverge somewhat, considering the symptom frequency and duration to be more inclusive and specific for IBS (differences between the Rome II and III criteria are described in the table below). This is somewhat complicated by discrepancies in the diagnostic criteria and the IBS questionnaire module. The questionnaire was mainly developed for research and is not a diagnostic tool, because IBS still remains a clinical diagnosis.

Table 1 The Rome Criteria for IBS

Rome II & III Criteria for IBS[17,24,25]	**Symptoms**	**Frequency**	**Duration**	**Onset of symptoms**
1. *Cardinal symptom*	Abdominal pain or discomfort	At least once per week (aR II, pR II and pR III)* At least 3 days per month (aR III)	Last 3 months (aR II and pR II and aR III)** Last 2 months (pR III)	Within last year (aR II) Within last 6 months (aR III) Not defined in Pediatric Rome III
2. *Associated bowel symptoms (≥2)*	a) Improvement with defecation b) Onset associated with changes of stool frequency c) Onset associated with changes of stool form(appearance)	Sometimes or more frequent***		
3. *No evidence of organic causes*				

aR= adult Rome Criteria for IBS; pR = pediatric Rome Criteria
*frequency not defined in the adult Rome II criteria for IBS, but in the Rome II IBS questionnaire module.
**duration of symptoms defined as last 3 months in the adult Rome II questionnaire module but as at least 3 months during the prior 12 months and not necessarily consecutive months in the adult IBS II criteria.
*** only stated in the pediatric Rome III Criteria, but also in the adult Rome II and III questionnaire module.

1.2. Prevalence of abdominal pain and IBS

Abdominal pain is reported to be the most common type of chronic pain among children in pre-pubertal age, with prevalence up to 25%.[8] The frequency of abdominal pain is described as stable from childhood to adolescence, whereas musculoskeletal pain and headache increase during adolescence and are the most frequent chronic pain conditions among both adolescents[26] and adults.[27-30]

IBS is considered to be the most common cause of abdominal pain in both pediatric and adult abdominal pain patients.[8,31-34] The prevalence of IBS in adults ranges from 2% to 25% in Western countries,[32] and some studies have demonstrated an increasing incidence of

FGIDs.[35] However, comparing previous epidemiological IBS studies is difficult due to different classification criteria, which contributes to the inconsistency of IBS prevalence estimates.[32,36] In a Norwegian population-based study, Vandvik et al. found the prevalence of IBS among adults to be 8.4 % (Rome II criteria).[37]

Hyams et al. found an increasing IBS prevalence from middle to high school students (8% to 17%), indicating that IBS is less common among younger children than adolescents, but the frequency of abdominal pain was similar in both age-groups.[33] In a recent study of prepubertal children in Germany, the IBS prevalence was 4.9%, but further epidemiological pediatric IBS studies are lacking in the Western world. Somewhat higher IBS prevalence (about 20%) is reported for adolescents in Asian countries.[38,39]

IBS is more common among female adults,[11] and abdominal pain is more common among adolescent girls.[8] Sex- related differences in the prevalence of abdominal pain are less consistently found among children before puberty.[8]

1.3. Comorbidity in abdominal pain and IBS

Comorbid chronic pain disorders are prevalent in patients with IBS, with fibromyalgia reported to be one of the most frequent (26% to 65%).[40] In a systematic review, psychiatric illness—mainly anxiety, depression and somatization disorders—is reported to be common among patients with IBS (54% to 94%).[41] Many of the studies examined severe patients with IBS seen in hospitals or outpatient clinics, and it is therefore questionable if these results are representative for most patients with IBS, who are never seen by a specialist. Still, a population-based Norwegian study by Vandvik et al. found both negative mood symptoms and multiple musculoskeletal pain to be more common in IBS than in controls (25% vs. 9% and 20% vs. 7% respectively).[37] Less is known about the strength of these associations among pediatric patients with IBS, but several studies of children with recurrent abdominal pain described increased prevalence of psychological and behavioral problems.[42-45]

The difference mentioned above in psychiatric comorbidity between patients with IBS and what is reported in population-based IBS studies may indicate that the somatic symptom burden is dose-dependently associated with the negative mood symptoms. Further, how the multiple somatic and psychiatric symptoms in IBS are related remains incompletely understood. A few studies of adult patients with FGIDs have shown an association of the number co-existing FGIDs and the abdominal pain intensity with degree anxiety and

depression.[46,47] Negative mood symptoms in IBS and other FGIDs therefore seem to be associated with the somatic symptom load in these patients. The significance of comorbid pain in this association is to our knowledge unclear. But there are some reports of an association between the number chronic pain sites and anxiety and depression among individuals with chronic pain in general.[48,49] We could for the same reason anticipate that the distribution of chronic pain is also related to anxiety and depression among individuals with IBS, but the literature provides no evidence for this interpretation. Further, how the different and specific abdominal pain dimensions and bowel symptoms in IBS are associated with negative mood is to our knowledge not fully understood. Hypothetically, both the specific bowel symptoms in IBS and the different abdominal pain dimensions (e.g., abdominal pain intensity, distribution, frequency, and duration) may be stressors triggering anxiety and depression symptoms.

1.4. The impact of abdominal pain and IBS

Reduced quality of life in patients with IBS compared to healthy individuals is reported in several studies, as are also increased use of healthcare services and impairments in daily life in both adult and pediatric patients with IBS.[43,50-52] These associations are also reported to be related to degree gastrointestinal symptoms, comorbid somatic symptoms, and psychological factors in patients with IBS. Abdominal pain is reported to be the most frequent chronic pain causing school absence among children and adolescents.[50] In the same study, pain intensity, rather than duration and frequency, was the most important pain feature affecting quality of life among the children and adolescents.

Also, health-related costs of IBS are reported to be related to comorbidity in IBS but also to the severity of IBS symptoms.[53,54]

1.5. Pathophysiological mechanisms of abdominal pain and IBS

1.5.1. The brain-gut model

FGIDs are by definition characterized by the absence of evidence of structural, metabolic, or biochemical abnormalities in routine clinical diagnostics. This does not necessarily exclude possible organic causes but simply reflects the fact that such factors are not detectable with currently available clinical diagnostic tools. There is increasing evidence that in at least subgroups of patients with IBS, the disorder has an organic etiology, although this is still

somewhat controversial. One example is the subset of patients with IBS with reduced tolerance to poorly absorbed carbohydrates, such as fructose, lactose, galactans, and fructans,[55] which is possibly related to alterations of the bacterial gut flora.[56] Alterations of the gut flora, primarily colonic flora, may be post-infectious after a resolved acute gastroenteritis.[57,58] The mechanisms are still incompletely understood, and some researchers claim that preexisting psychological characteristics are important in the development of post-infectious IBS.[59,60] This exemplifies the complex interplay between psychological and biological factors and central nervous system and local gut factors, in what has become known as the brain-gut model in IBS. Overall, there is increasing evidence of a brain-gut model in IBS and other FGIDs, where biological, psychological, and social factors contribute and interact in triggering and maintaining the different abdominal and bowel symptoms.[14,16,61-63] The brain-gut model illustrated in Figure 1 describes plausible peripheral and central mechanisms in IBS and the communicating pathways. Whereas some factors and mechanisms are most likely to be present in abdominal pain patients in general, others are more specific to IBS.[13,14,34]

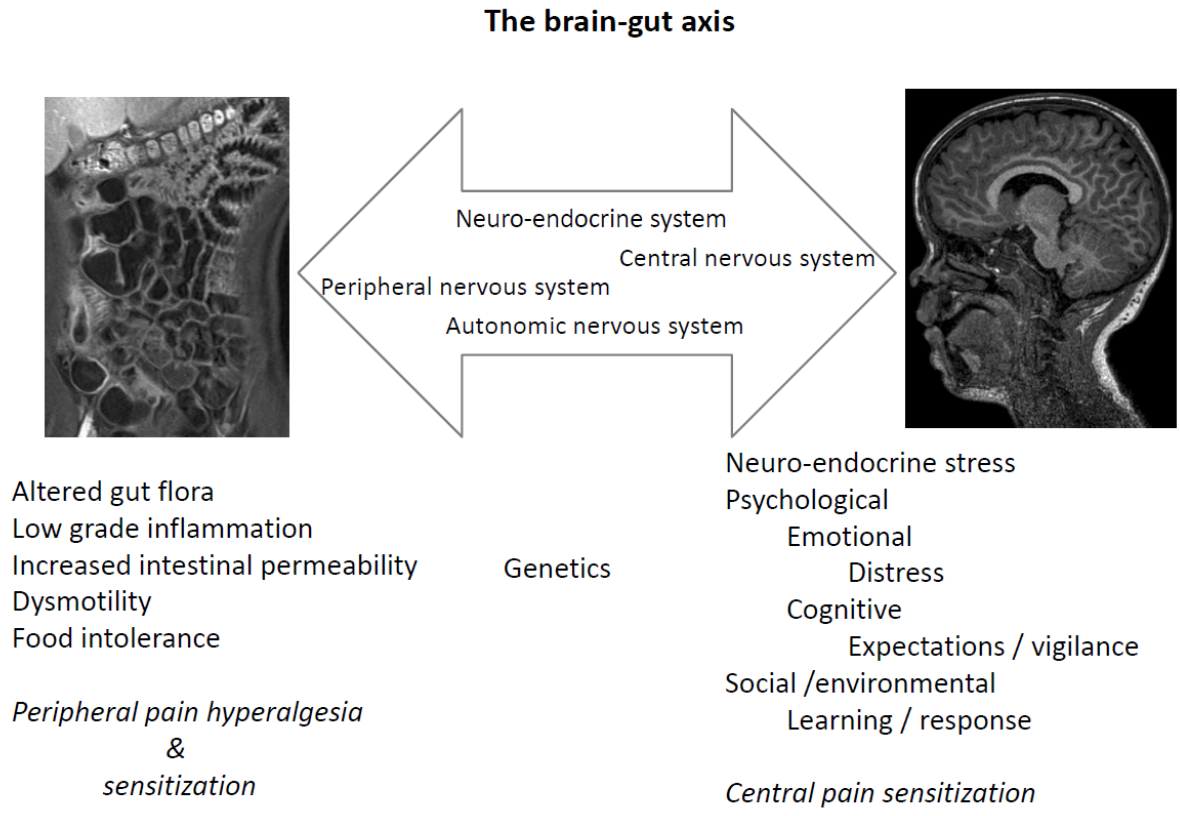


Figure 1 The brain-gut model in IBS.

1.5.2. Increased pain sensitivity and pain sensitization mechanisms

Visceral hyperalgesia is found to be a "diagnostic marker" of IBS, with high sensitivity and specificity.[64] Still, increased visceral sensitivity is not evident in all patients with IBS compared to healthy controls and has yet not become a routine clinical diagnostic tool. Visceral hypersensitivity is manifested as lowered visceral pain thresholds and increased pain intensity in response to balloon extension of the rectum.[64] The degree of visceral hypersensitivity is found to be related to the severity of IBS symptoms[65] and also to comorbid psychological factors.[66] Whether visceral hyperalgesia is a local gut phenomenon or is related to mechanisms in the central nervous system is debatable. Some IBS studies have found evidence of increased pain sensitivity in other tissues and in body areas other than in the visceral organs,[67] but whether or not this is related to comorbid chronic pain conditions in IBS remains unclear.[68,69] To what degree widespread hypersensitivity in IBS is related to psychological factors or is independently related to the primary condition is also not fully understood.[70,71]

Less is known about the relationship between pain sensitivity and IBS and other FGIDs among children and adolescents. Some studies of children with AP and IBS have demonstrated increased visceral pain sensitivity comparable to that found in adult patients.[72-74] Little is known about widespread pain sensitivity among pediatric patients, and the results of the few studies of children with RAP are somewhat conflicting.[75-78]

Pain sensitivity is the relationship between the intensity of a noxious stimulus and experienced pain, and it reflects the "gain" of the entire nociceptive processing system. This relationship can be quantified by applying well controlled experimental pain stimuli and observing the individual's pain response (i.e., pain rating). As for pain in general, there is great inter-individual variation of measured pain sensitivity and stimuli that lies well beyond the tolerance limit of some individuals and may lie well below pain threshold of others.[79] Pain sensitivity is dependent on demographic factors (e.g. sex, age, and ethnicity), psychological factors, and as discussed below, it is associated with chronic pain symptoms.[80] Pain sensitivity is poorly correlated across different pain modalities (e.g., heat and pressure pain).[81] This lack of correlation cannot be dismissed as mere measurement error, since the reliability of experimental pain sensitivity tests is generally high.[82-84] Thus, it would seem that

pain sensitivity is modality specific, which raises the question of which modalities are relevant to which types of clinical pain.

How chronic pain, pain sensitization, and hyperalgesia are related is still not fully understood. Pain sensitization is believed to be a consequence of repeated and sustained pain sensations, for which there is some evidence from animal models and from experimental pain sensitivity research with human volunteers.[85,86] There is less clinical evidence for these theories, but some studies have shown that increased pain sensitivity also predicts higher levels of post-operative acute pain and chronic pain.[87] The causal directions still remain unclear; possibly the relationships are bidirectional and/or there are shared pathophysiological mechanisms. Figure 2 below shows an hypothetical model of possible relationships between chronic pain, pain sensitization, and increased pain sensitivity.

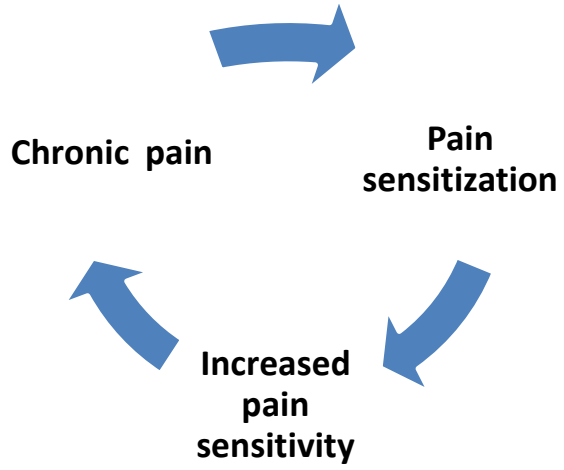


Figure 2 Possible relationships between chronic pain, pain sensitization, and increased pain sensitivity.

Reduced pain inhibition and /or increased pain augmentation in IBS has been reported at different anatomical levels of the nervous system: in the peripheral afferent nerve fibers, spinal and supra-spinal levels in the central nervous system, and in addition the autonomic nervous system.[61,88]

Intestinal immune activation and increased intestinal permeability is described in IBS and possibly leads to persistent peripheral neural input and peripheral sensitization.[62] There is evidence of spinal pain sensitization in IBS, with greater pain hypersensitivity in the lower

extremities compared to upper extremities.[89] Further, local rectal anesthesia has been shown to reduce not only visceral but also somatic pain sensitivity in the lower extremities in patients with IBS, whereas similar effects not were seen in healthy controls.[90] This supports theories of spinal pain sensitization in IBS but does not rule out the importance of supra spinal mechanisms.

The endogenous pain modulation system is found to be influenced by activity in the autonomic nervous system, neuroendocrine system, and neuroimmune interactions.[61,62,88] Altered autonomic reactivity has been reported in a subset of patients with IBS during symptom flares, with changes in both sympathetic and parasympathetic tones that affect the enteric nervous system and gut motility.[91,92] Vagal stimulation may attenuate pain both centrally and peripherally,[93] while efferent sympathetic fibers participate in immune activation in the gut.[94] Corticotropin-releasing hormone, produced in the hypothalamus, has a key role in the response to stress and pain in addition to inhibiting gastric emptying and stimulating colonic motor function.[95]

Supra-spinal modulation of pain is a dynamic balance between inhibition and facilitation mechanisms, located in the brainstem and higher pain centers.[85,88] Central pain processing communicates neuroanatomical bidirectionally with cognitive, emotional, and autonomic brain areas.[61] Central pain processing is complex; we have increasing knowledge of which brain areas are involved, but the physiological mechanisms are still not fully understood.

As described above, increased visceral and somatic pain hypersensitivity in patients with IBS has been studied employing simple experimental stimulus paradigms. However, these paradigms reveal little about the actual mechanisms contributing to hypersensitivity. Several dynamic experimental pain methods have been developed to explore the endogenous pain modulation processes. Pain sensitization is revealed by repetitive painful stimuli, referred to as temporal summation of pain. As compared to healthy controls, increased temporal summation of pain has been found in several chronic pain patient groups, such as in persons with fibromyalgia.[96] An intensely painful stimulus like in the Cold-pressor test has been shown to reduce pain applied at other body sites (= conditioned pain modulation). In experimental pain studies, conditioned pain stimulation has shown that patients with IBS have reduced endogenous pain inhibition or increased pain facilitation compared to healthy individuals.[77,97] The increasing number studies of FGIDs patients that combine experimental pain with functional brain imaging are providing new insights and a better understanding.[98]

Primarily quantitative differences in activity levels in brain areas are seen in IBS as compared to healthy individuals.[99]

Despite the increasing number studies of pain sensitivity and pain modulation in IBS and to some degree in other FGIDs, most of the studies are small, and none have been conducted in representative population-based samples. Furthermore, a majority of the studies were limited to premenopausal female patients. It is therefore questionable whether these patients are representative for most individuals with IBS symptoms, many of whom never seek healthcare for their abdominal symptoms[11,100] and others are seen only in primary care.[101,102] Studies have shown that the majority of IBS patents are followed by their family doctors and not by gastroenterologists.[11] Further, the extent to which previous results are representative for males, older persons, and children and adolescents with IBS symptoms is uncertain.

1.5.3. Local gut mechanisms

As discussed above, there is evidence of local gut mechanisms of IBS.[15] Increased intestinal permeability, sub-clinical inflammation, and altered gut flora are possible local factors that contribute to triggering and maintaining IBS symptoms.[103,104] These local factors may also contribute to intestinal and colonic dysmotility in IBS.[12] Further, they are thought to be associated with systemic mechanisms through immunological processes and via the peripheral and central nervous system.[62] There are still gaps in our knowledge of how the peripheral gut factors and the central nervous mechanisms in the brain –gut axis are connected in IBS. For instance, the importance of peripheral gut factors in visceral hyperalgesia versus sensitization mechanisms in the central nervous system is not fully understood.

Subsets of patients with IBS have reduced tolerance for fermentable carbohydrates. Increasing evidence shows that a subset of patients with IBS improve by eliminating or restricting dietary intake of fermentable oligo-di-mono-saccharides and polyols (FODMAPs); a low FODMAP diet may reduce both abdominal pain and the associated IBS bowel symptoms.[55,105]

Other local factors are related primarily to bowel movement symptoms in IBS rather than to abdominal pain symptoms. Some of the patients with diarrhea-predominant IBS have been

found to have increased levels of malabsorbed bile acids in the colon.[106] High levels of colonic bile acids are thought to be a contributing cause of diarrhea in this subset of patients with IBS. Elevated levels of enteroendocrine production of serotonin are also reported among subsets of patients with IBS with prominent diarrheal symptoms.[15,107]

1.5.4. Psychosocial mechanisms

As mentioned above, IBS and AP are strongly associated with a number of psychosocial comorbidities. Anxiety, depression, and somatization are the most common psychiatric comorbidities. Some researchers consider these factors to be predictors of abdominal pain and IBS,[108-110] but it is debatable whether the relationships are causal, and if so, what the direction of the causation is. Although some longitudinal cohort studies have been conducted, causal interpretations are often difficult to make due to limitations in study design. Overall, the possible causal relationships are debatable and to what degree the symptoms have shared pathophysiological mechanisms is still not fully understood.[14,63] Therefore, a dualistic psychosomatic understanding of IBS as having a primarily psychiatric origin is being increasingly replaced by the biopsychosocial brain-gut model described above.

Adverse events in early life, such as child abuse, have been cited as possible predictors of abdominal pain and IBS later in life,[111,112] but as supporting evidence comes from cross-sectional studies, where results may be affected by recall bias, the results are inconclusive. Contradictory evidence comes from another large longitudinal cohort study, where adverse childhood events were not found to predict IBS later in life, whereas psychopathology was found to be a significant predictor of IBS.[109] Similar findings are reported in other studies, and for other psychological factors, such as somatization.[110]

Some researchers believe that parental factors, including parental psychological and physical illness, are predictors of childhood abdominal pain and IBS.[113-115] Again, the results of most of these studies can only be interpreted as associations and not as clear etiological relationships, due to limitations in the study design.

1.5.5. Heritability of IBS and abdominal pain

Several studies have also found familial aggregation of abdominal pain and IBS, possibly indicating that both environmental and genetic factors are of importance.[116,117] The magnitude of the heritability is considered moderate, as reported in a systemic review of

chronic pain twin-studies.[118] Further, an increasing number of candidate genes have been found in a subset of patients with IBS. More than 60 genes have been hypothesized to genetically predispose to IBS, but their clinical importance is still unclear.[119] Molecular-biological studies of IBS are difficult for several reasons. Identifying candidate genes is problematic due to the heterogeneity of the IBS phenotype, but specific gene variants are found in subsets of patients with IBS, such as gene variants linked to immunological processes of the gut in some patients and gene variants linked to serotonin signaling in the brain-gut axis in others.[119]

2. STUDY QUESTIONS AND AIMS

There is still conflicting data regarding the presence of widespread hyperalgesia in IBS. Specifically, it is not clear whether this association is independent of comorbid chronic pain and psychological distress symptoms. Furthermore, the available evidence is based on clinical convenience samples, and it is not known whether the findings are valid for the general adult and adolescent population.

- The main aim of the study was therefore to compare measured pain sensitivity among individuals with and without IBS symptoms in representative adult and adolescent samples drawn from the general population, controlling for differences in prevalence of comorbid chronic pain and psychological distress (Papers 1 & 2 in this dissertation).

It is well established that IBS is strongly associated with depression and other forms of negative affect. However, it is not known what specific pain dimensions (e.g., pain distribution, duration, intensity, and frequency) are of importance in this association. Furthermore, it is not known whether the bowel habits that distinguish IBS from abdominal pain in general are associated with negative affect independently of abdominal pain.

- The second aim was therefore to describe how different abdominal pain dimensions and associated bowel habits were associated with depression among adolescents (Paper 3 in this dissertation).

3. MATERIALS AND METHODS

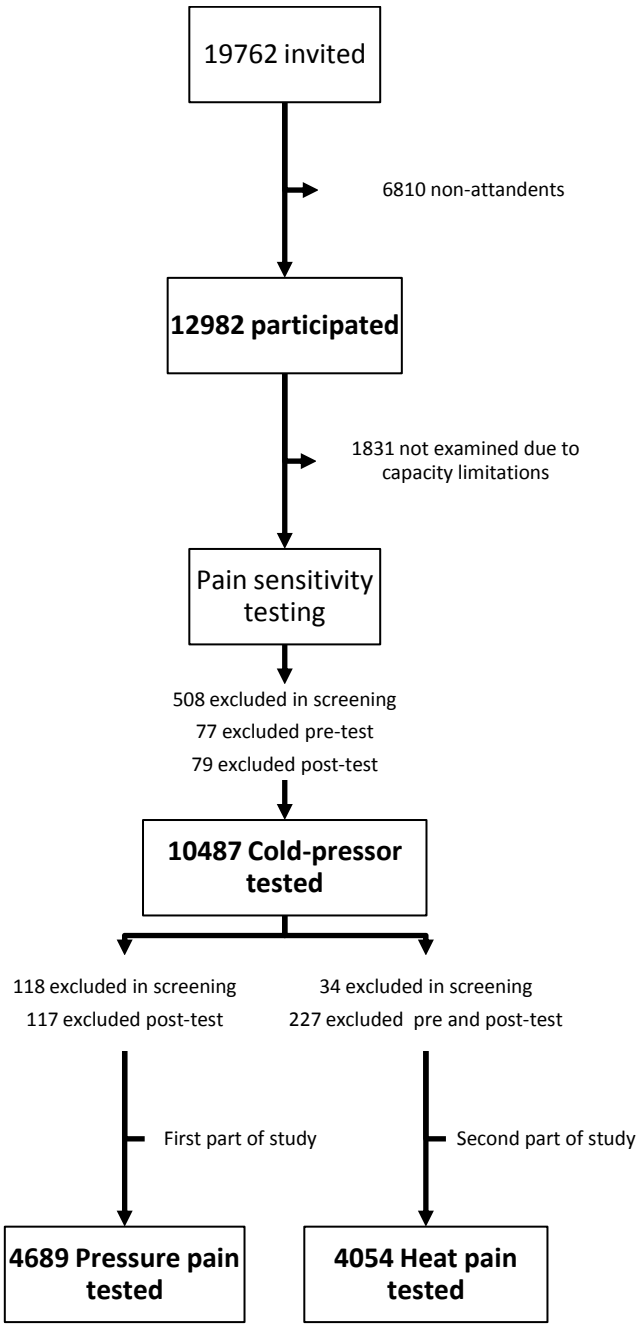
3.1. Sample

The Tromsø Study was initiated in 1974 to identify risk factors of cardiovascular diseases, which were high-prevalent in this area of Norway. Over time the Tromsø Study was expanded to include a wide range of projects related to other lifestyle-associated diseases and common health problems in the population. Since the start, six study-waves have been conducted with adults in the municipality of Tromsø. The study is longitudinal and follows adults participating in more than one wave as well as new invited participants in each subsequent study wave.

The last adult study was conducted in 2007-2008 (the sixth wave). Of the 19,762 adults invited to participate, 66% attended (n = 12 682, median age 58 years (range, 30-87 years) and 53.4% women). The sample was a combination of cohorts from previous study waves and newly invited adults (Jacobsen et al.[120]). The participants were asked to fill out a health questionnaire and to participate in extensive examinations, with medical and physical measurements that also included measurements of pain sensitivity (see flow chart of the study, below).

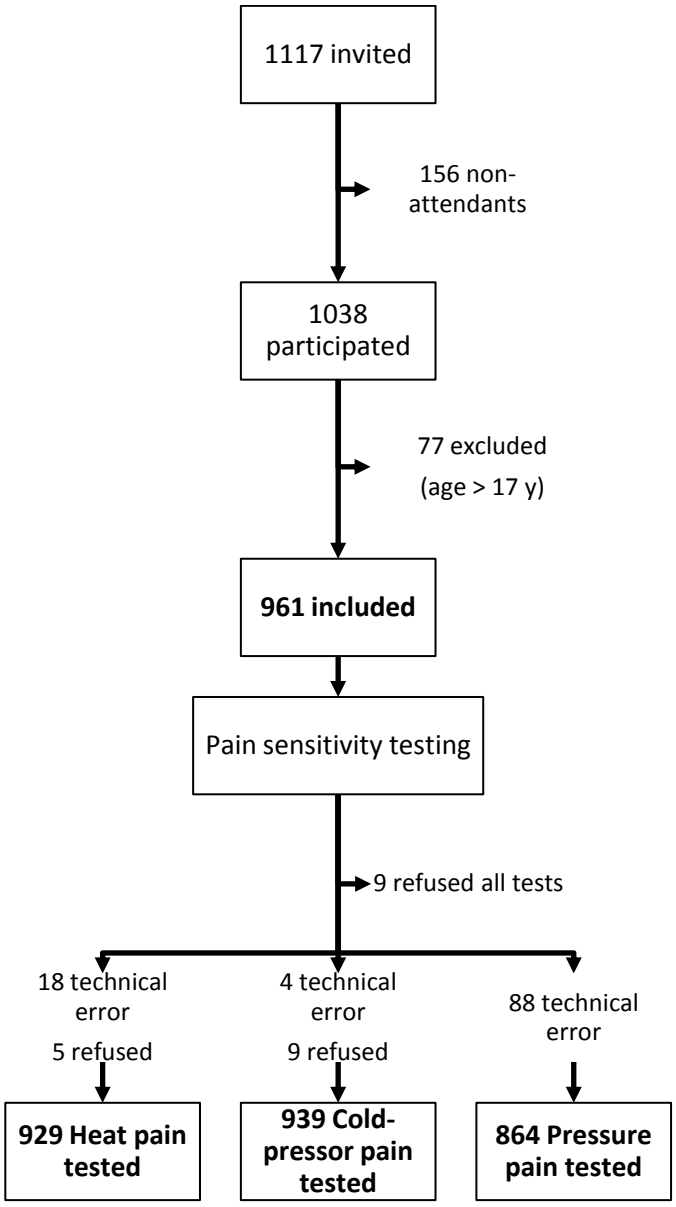
In 2010-2011 the Tromsø Study was expanded to include all adolescents enrolled in the first year of high school (this part of the Tromsø Study was named Fit Futures). Students in both academic and vocational student programs at all five high schools in the study area participated (n = 1,117, with 92.9% response rate n = 1038). The median age of the participating students was 16 years (range, 15-28 years), and 48.9% were girls. Students older than 17 years of age were excluded in the analyses in the dissertation study (n = 77).

Figure 3 Flow chart of the study: Pain sensitivity testing in the adult study



Excluded, pre-test: Capacity limitations, technical failure, medical reasons or lack of comprehension.
Excluded, post-test: Invalid tests due to technical and procedural failures or lack of comprehension.

Figure 4 Flowchart of the study: Pain sensitivity testing in the adolescent study



3.2. Informed consent

Both the 6th wave of the Tromsø Study and the Fit Futures Study were approved by the Norwegian Social Science Data Services and the Regional Committee for Medical and Health Research Ethics, Health Region North. All participants gave their written informed consent before inclusion in the study. For participants younger than 16 years, additional consent was given by the parent.

3.3. Questionnaire-based measurements and case definitions

The questionnaires in the adult study were paper-based, whereas the adolescents in the Fit Futures study filled out the health questionnaire electronically, using study-laptops.

Self-reported symptoms were recorded in the study questionnaires. Self-reported diagnoses were not included, and no clinical diagnostics were conducted to confirm the symptom-based cases as described below.

3.3.1. IBS case definition: Adults

In a separate part of the questionnaire, participants who had abdominal discomfort or pain during the last year reported if they had the symptoms weekly or more frequently. In the next follow-up questions they reported whether defecation relieved the abdominal discomfort or pain (1) and if the abdominal symptoms were related to more frequent or seldom bowel movements (2) and to harder or looser stools (3).

Adults reporting weekly abdominal pain or discomfort during the last 3 months or more combined with two or more associated bowel symptoms (numbered 1 to 3 just above) were classified as IBS cases in accordance with the Rome II criteria.[121,122]

The participants were asked to rate degree of abdominal pain immediately before the IBS module. If they fulfilled the IBS criteria, the degree of abdominal pain in the IBS cases could be sub-classified as no, mild, or severe abdominal pain.

3.3.2. IBS and abdominal pain case definitions: Adolescents

The adolescent participants were asked in a separate gastrointestinal questionnaire module how often they had abdominal discomfort or pain the last 2 months. Participants that reported weekly or more frequent symptoms were asked to answer the related follow-up questions. In the subsequent questions they were asked to localize the abdominal discomfort or pain (above, around and/or below the belly button), how long the abdominal

symptoms normally last (1-2 hours, 3-4 hours, most of the day, or all the time), for how long the symptoms had lasted (1 month or less, 2 months, 3 months, 4-11 months, or one year or longer). They were then asked to report the average intensity of their abdominal pain, rated on a numeric rating scale (NRS) from 0-10, where 0 = no pain and 10 = the most intense pain imaginable.

Adolescent participants who reported abdominal discomfort or pain were then asked to report if the symptoms were related to the following bowel symptoms with a frequency of never/rarely, sometimes, or usually: if the discomfort or pain got better after defecation (1) and if the symptoms were related to more frequent or seldom bowel movements (2) and to harder or looser stools (3).

Adolescents were classified as IBS cases in accordance with the pediatric IBS III criteria[17] if they reported weekly abdominal pain or discomfort during the past 2 or more months and with two or more of the associated bowel symptoms at least 25% of the time (sometimes or more frequently). The adolescent IBS cases were further sub-classified according to their pain ratings as mild (NRS = 1-3), moderate (NRS = 4-6), and severe (NRS = 7-10) (none reported NRS = 0).

The study on abdominal pain dimensions and depression in adolescents (paper 3 in this dissertation) used a broad definition of abdominal pain so as to include as many participants as possible, enabling further subgroup classification with respect to pain frequency and duration and other dimensions of abdominal pain. Therefore, participants reporting abdominal pain during the past 2 months that occurred at least once a month were defined as abdominal pain cases.

3.3.3. Comorbid chronic pain case definition

In both the adult and the adolescent study, chronic pain was assessed by items in a separate section of the questionnaires. Participants were classified as having chronic pain if they responded 'yes' to the question, "Do you have persistent or frequently recurring pain that has lasted for 3 months or more?"

Participants who responded yes to the initial question completed follow-up questions, including a checklist of body sites where they experienced pain. Since this broad question might include abdominal pain, follow-up questions on pain location were used to identify

subjects with non-abdominal chronic pain. Thus, subjects reporting pain lasting 3 months or longer at any non-abdominal site (i.e., head, yaw, neck, back, shoulder, arm, hand, hip, leg, foot, genitalia, or skin) were classified as having comorbid chronic pain.

3.3.4. Psychological distress and depression case definitions

Psychological distress symptoms were assessed with the 10-item version of the Hopkins Symptom Check List (HSCL). HSCL is a screening tool for detecting negative mood symptoms suited for epidemiological research, also among adolescents, and has been validated against clinical diagnostic depression and anxiety tools.[49,123-125] Subjects with a mean score of 1.85 or higher were classified as having psychological distress. This cut-off has previously been shown to have a sensitivity of 89% and specificity of 98% with respect to detecting anxiety or depression compared to the more extensive 25-item version of the HSCL.[126] If data were missing for less than four of 10 questions, missing values were replaced by the mean score of the remaining answers; otherwise, the HSCL score was set to missing.

In the adolescent study additional assessment of depression was made with the Short Mood and Feeling Questionnaire (SMFQ). SMFQ is a 13-item screening tool for depression that has been validated in both clinical samples and in the general population.[127,128] SMFQ sum scores ≥ 11 have a sensitivity of 86% and specificity of 87% for detecting clinical depression in adolescents.[129] Consequently, participants scoring above this cut-off were operationally defined as cases with depression.

3.4. Measurements of pain sensitivity

The study flowcharts and in the Table 2 below provide an overview of enrollment in the different pain sensitivity tests in the adult and adolescent studies. The testing was conducted by a trained nurse, using a data-assisted protocol. Prior to each test, the test procedure was described, and the instructions for threshold, tolerance, and pain intensity rating responses were given as appropriate for each test (see below). Pain intensity ratings in the adult study were given on a 0 to 10 numeric rating scale (NRS), with the anchors “no pain” and “the most intense pain imaginable.” Since the participants’ hands were occupied during the testing, participants made their ratings by calling out a number from 0 to 10. In the adolescent study, pain intensity ratings were given on a computerized visual analog scale

(VAS) controlled by a large trackball, with the same anchors as for the NRS. Instructions for use of these scales were adapted from Price et al.[130]

In the adult study, participants were asked to complete tests of pain sensitivity, which included the cold-pressor test and either pressure pain threshold (first half of study) or heat pain threshold (second half of the study). In the adolescent study, all the participants were tested in following order; heat, pressure and cold-pressor pain sensitivity measurements.

Table 2 Overview of the Pain Sensitivity Testing

Pain modality		**Body site**	**Adults**	n	**Adolescents**	n
Cold-pressor				10487		939
		Hand + wrist				
	Endurance		X		X	
	Pain ratings		X		-	
Heat				4054		929
		Underarm				
	Threshold		X		X	
	Tolerance		_		X	
Pressure				4689		864
		Finger	X		X	
		Shoulder	_		X	
	Threshold		_		X	
	Supra-threshold		X		_	
	Tolerance		_		X	

n = number of valid tests included in the statistical analyses

3.4.1. Cold-pressor test

Cold-pressor pain was induced using a 3°C water bath (Julabo PF40-HE, JULABO Labortechnik GmbH, Germany) connected to a 13 L external plexiglass container with a flow of 22 L/Min. The participants submerged their left hand and wrist in the cold water in the plexiglass container as long as they were able to, up to maximum 105 seconds. Time to withdrawal of the hand was recorded (tolerance limit).

In the adult study, NRS pain intensity ratings were obtained 4 seconds after the beginning of the test and every 9 seconds thereafter until hand withdrawal. In the adolescent study, VAS pain intensity ratings were obtained in a similar way, but as these data were not analyzed in the papers included in this dissertation, the details are omitted.

3.4.2. Heat pain tests

Heat-pain threshold and tolerance (adolescents only) were tested using a MEDOC ATS somatosensory stimulator (MEDOC Ltd, Israel) with a 30x30 mm thermode. Stimuli were applied to the volar surface of the right forearm. Stimulation started from a baseline temperature of 32.0°C and increased by 1°C/s, with an upper safety limit of 50.0°C. For pain threshold measurements, participants were instructed to press a button when the sensation changed from warmth to pain. Upon pressing the button, the temperature was registered and the thermode temperature returned to baseline at a rate of 8°C/s. This procedure was repeated three times; the first measurement was discarded and second and third measures were averaged. In the adolescent study, threshold measurement was followed by assessment of pain tolerance. Using the same stimulus parameters, participants were asked to press the button at the maximum tolerable pain level; they were informed about the preset safety-limit at 50.0°C. Heat pain tolerance was measured twice, and the highest temperature was recorded.

3.4.3. Pressure pain tests

Pressure pain threshold and tolerance (adolescents only) were tested using a hand-held algometer (Somedic AB, Sweden) with a circular probe of 1 cm^2. Starting at 0 kPa, pressure was increased by 30 kPa/s.

Among the adolescents, pressure-pain threshold and tolerance were recorded with the adolescent pressing a button when the sensation changed from non-painful pressure to pain

and at a maximum tolerable pain level, respectively (limit of 1000 kPa). Pressure was applied to the cuticle of the ring finger nail of the right hand and on the midline of the right trapezius muscle and in shoulder height. The threshold measurements procedure was repeated three times, followed by tolerance measurements, repeated twice. For each site, the second and third threshold measure was averaged, and the highest tolerance measurements were recorded.

In the adult study, only the finger site test was conducted with measurements of supra-pressure pain thresholds. The participants were instructed to press a button when pain intensity reached NRS = 5, upon which the pressure was stopped. Starting at 0 kPa, pressure was increased by 30 kPa/s up to a maximum of 800 kPa. As with the adolescents, the procedure was repeated three times for each subject, and the average of the last two measurements was used in the analysis.

3.5. Statistical analyses

The statistical methods are described in each separate paper (papers 1, 2, and 3 in this dissertation), but the main statistical methods are listed below. The analyses were done using SPPS version 18-21. Results are reported with 95% confidence intervals, and results with $p < 0.05$ are reported as statistically significant.

Table 3 Overview of the Statistical Analyses

Main outcome variable	**Main analyses**	**Uni-variable analyses**	**Multi-variable analyses**	**Post-hoc analyses**	**Analyses of confounding**	**Analyses of interaction effects**
Continuous						
Normal distribution **Pain thresholds and pain ratings**	IBS vs. control	Student t-test	ANOVA and ANCOVA	Pair-wise comparisons IBS pain subgroup vs. control	Adding co-factors in a stepwise approach	Visually and full factorial model
Skewed distribution **Pain tolerance**	IBS vs. control	Mann-Whitney U test				
Skewed distribution with censored data **Pain tolerance**			Multiple Cox regression	IBS pain subgroup vs. control	Adding co-factors in a stepwise approach	Visually and including IBS - sex and IBS - co-variable interaction terms
Categorical						
Dichotomous **Depression (SMFQ ≥ 11)**	IBS and AP vs. control	Chi-square test	Multiple logistic regression	Within AP symptom subgroups	Stepwise approach	Including interaction terms in the multiple regression analyses

ANOVA = analyses of variance, ANCOVA = analyses of co-variance. SMFQ = Short Mood and Feeling Questionnaire.

The Cox regression model was chosen to analyze all pain tolerance results in paper 1 and paper 2 because of the relative high number of individuals that did not have tolerance levels

below the preset maximum test limit (105 s in the cold-pressor test, 50.0°C in the heat pain test, and 1000 kPa in the pressure pain tolerance test). The same approach was applied to analyze the supra-threshold levels in the pressure pain test with the adult participants (limit at 800 kPa).

In paper 1, sex, age, comorbid chronic pain, and psychological distress were entered as co-factors in the analyses of pain sensitivity in IBS and in the analysis of IBS pain subgroups versus controls. Since age was non-linearly related to pain sensitivity, age was categorized in four age groups (30-49, 50-59, 60-69, and 70-87 years). In the stepwise analyses (Cox regression, analysis of variance) age and sex were included as co-factors in the first step. In the second step comorbid chronic pain and psychological distress were added.

In paper 2, the same statistical approaches were conducted as described in paper 1, but age was not included as a co-variable, since there was virtually no variance in age variability among the adolescents (15-17 years old), and no differences in mean age between IBS and control groups.

In paper 3, associations between depression and IBS and abdominal pain were analyzed using stepwise logistic regression, adjusting for differences in sex, parental educational level (< college or ≥ college), and comorbid chronic pain in the second step. Finally, analysis of associations between depression and abdominal pain dimensions were performed for individuals with abdominal pain only. This analysis was performed in two steps: First, the relationship between depression and each symptom dimension was tested individually, using logistic regression and controlling for sex. Next, all symptom dimensions that were statistically significant in the first analysis were entered in a multivariate logistic regression. This two-step approach was used due to the large number of predictors tested in a relatively small sample.

4. SUMMARY OF THE RESULTS

4.1. Paper 1

- Among the adults, 5.3% reported IBS symptoms (Rome II criteria).
- Adults with IBS had lower heat pain thresholds, lower cold-pressor pain tolerance, and higher pain ratings in the cold-pressor test compared to controls.
- The associations between increased pain sensitivity and IBS were independent of sex, age, comorbid chronic pain, and psychological distress symptoms.
- The associations were related to degree abdominal pain, with the severe abdominal pain IBS cases being the most pain sensitive. The associations were also found to be independent of the co-factors listed above.
- There was no significant difference in the pressure pain thresholds between IBS cases and controls.

4.2. Paper 2

- Among the adolescents, 8.2% reported IBS symptoms (Rome III criteria).
- Adolescents with IBS had lower heat pain thresholds and pressure pain thresholds in two body sites than controls.
- The associations between pain thresholds and IBS were independent of sex and comorbid chronic pain, but only heat pain and the overall pain threshold sum score were significant after further adjustment for psychological distress.
- The associations were related to degree of abdominal pain in the heat pain threshold test and the overall pain threshold sum score, with severe abdominal pain IBS cases being the most sensitive. The associations were also found to be independent of the co-factors listed above.
- There was no pain tolerance difference between IBS cases and controls.

4.3. Paper 3

- Among the adolescents, 27% (n = 259 of 961) reported abdominal pain monthly or more often, and 30% of these (n = 77) fulfilled the IBS Rome III criteria.

- The prevalence of depression was 20.5% among adolescents with abdominal pain compared to 10.5% in controls.
- The odds of depression were similar for abdominal pain and IBS cases compared to controls (OR = 2.5 vs. 2.4 respectively), after adjustments for sex, comorbid chronic pain, and level of parental education.
- In the multivariable regression analyses within the abdominal pain group, the following symptom dimensions were independently associated with depression: severe abdominal pain intensity, widespread abdominal pain, and presence of comorbid chronic pain.
- Sex, parental education, and other abdominal pain symptom dimensions, including bowel symptoms that distinguish IBS from abdominal pain, were not independently associated with depression.

5. DISCUSSION

5.1. General discussion: Biopsychosocial understanding of IBS and abdominal pain

We have in this work demonstrated that individuals with symptoms of IBS have increased widespread pain sensitivity (paper 1 and 2). Furthermore, we have found that degree abdominal pain, diffuse abdominal and widespread pain distribution are important pain dimensions in the association with depression among adolescents with abdominal pain (paper 3). All these results fit in to a biopsychosocial understanding of both IBS and AP, with chronic abdominal pain symptoms associated with both widespread hyperalgesia and negative affective symptoms.

Finding widespread pain sensitivity indicates that central nervous mechanisms are of importance in IBS, most likely in addition to the peripheral mechanisms as discussed in the introduction above. Our findings not only strengthen previous reports on widespread pain sensitivity in patients with IBS but also are importantly more generalizable due to the population-based study design.

Clearly, interpretations concerning causality are inappropriate in our project. Most likely the relationships among chronic pain symptoms, increased pain sensitivity, and negative affective symptoms are multidirectional, possibly with shared pathophysiological mechanisms. As described in the introduction above, many researchers believe that pain sensitization and increased pain sensitivity are consequences of chronic pain rather than causes, although there is also evidence of the opposite. Laboratory-based experiments on long-term or repeated pain stimulation of afferents (long-term potentiation and temporal summation studies) suggest possible mechanisms whereby persistent pain may lead to pain sensitization.[85] The fact that hyperalgesia predicts increased post-operative pain and development of post-operative chronic pain supports the opposite theory.[87] Further evidence is provided by a large prospective study where several measures of pain sensitivity were found to be associated with greater incidence of chronic temporomandibular joint pain.[131] As far as we know, there are no other comparable prospective studies that include pain sensitivity assessments and record chronic pain conditions other than temporomandibular joint pain. Therefore, the temporal relationships between chronic pain (including IBS and abdominal pain) and increased pain sensitivity are mostly unknown,

making causal inferences uncertain. Finally, the importance of psychological factors such as anxiety and depression in the association between IBS and pain sensitivity is still unclear[13,132], but possible relationships are illustrated in Figure 5 below and discussed further in the next section of the Discussion.

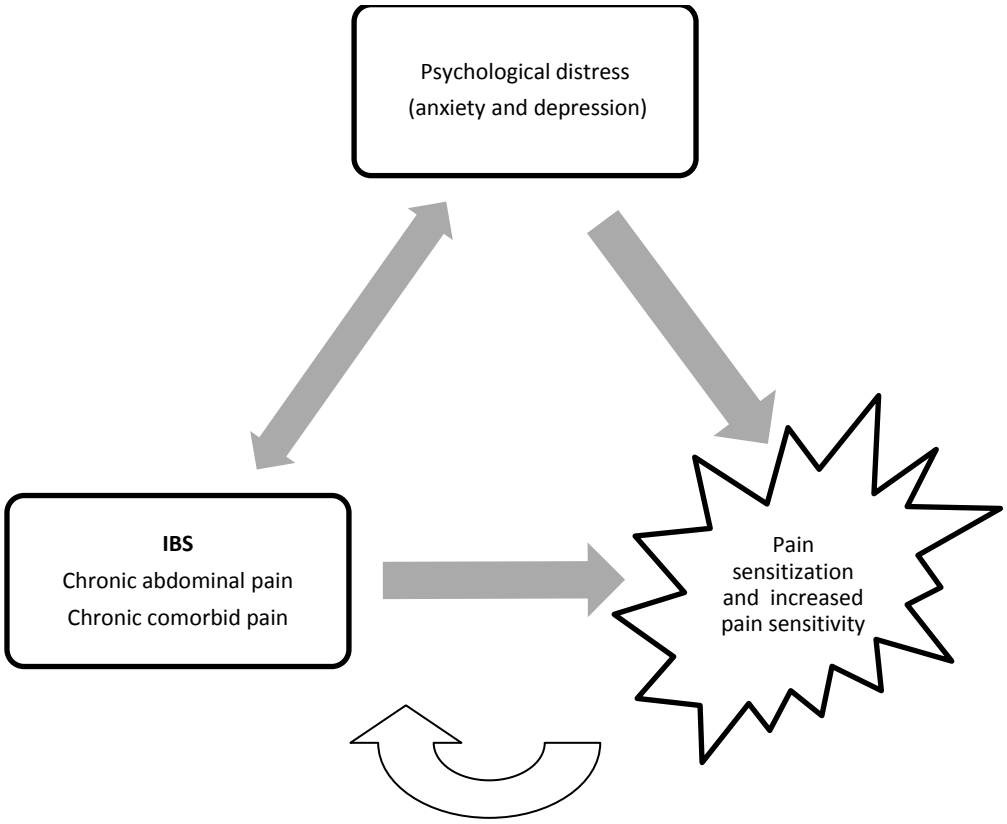


Figure 5 Possible relationships between chronic pain in IBS, psychological distress, and pain sensitivity. Hyperalgesia may hypothetically trigger and maintain chronic pain symptoms, as illustrated by the lower positive feedback arrow.

As discussed in the introduction of this thesis, there is evidence that several factors contribute to triggering and maintaining IBS symptoms rather than a single cause (the biopsychosocial model). None of these factors can be interpreted as causal factors in a strict sense, because none are mandatory. There is also no well-documented temporal relationship between the factors and IBS. Although the biopsychosocial model of IBS and FGIDs is highly adopted,[13,14,34,61,63] there are controversies concerning the importance of the different factors in the model as well the question as to whether the factors are independent of each other.

In the following sections, our study results will be discussed in the context of understanding IBS as a multifactorial condition, including the importance of increased pain sensitivity, comorbid chronic pain, and psychological distress and will outline how our findings contribute to previous research in this field.

5.2. Increased widespread pain sensitivity in IBS

All previous studies of pain sensitivity have been conducted with IBS patients recruited in hospitals or in specialized out-patient clinics.[67,89,133,134] The majority of studies included only premenopausal female patients with IBS. Furthermore, there is to our knowledge only one published pediatric IBS study on widespread pain sensitivity in a sample premenarchal girls, including 21 case and an equal number of controls.[77]

Overall, a majority of the studies with adults report evidence of widespread pain in patients with IBS,[67,69,97,132,135,136] whereas this was not found in the single pediatric study[77] and in one adult study.[133] The question is whether these results are representative and valid for the vast majority of individuals with IBS symptoms. A larger proportion of individuals with IBS symptoms never seek health care for their abdominal complaints.[11,100] Furthermore, the majority of patients with IBS that are seen by general practitioners have fewer IBS symptoms and less comorbidity than patients referred to specialist clinics.[101] Consequently, there is a risk of selection bias in previous studies, with the milder IBS cases not selected and not participating.

In contrast to in these case-control studies, we have documented increased pain sensitivity among individuals with IBS symptoms in large, representative samples drawn from the general population, including both sexes, adults and adolescents, and IBS cases with both mild and severe symptoms. Therefore, our study results, which are largely in line with previous findings, not only strengthen existing evidence of increased widespread pain sensitivity in IBS but also support generalization to a wider spectrum of individuals with IBS symptoms.

To what degree widespread hyperalgesia in IBS is a result of confounding with comorbid chronic pain conditions is unclear. This issue is of some importance, as increased pain sensitivity has been demonstrated in a number chronic pain conditions, such as fibromyalgia, that are common in patients with IBS. Some previous adult studies have included patients

with and without comorbid fibromyalgia in addition to healthy controls, with some reporting that IBS is independently associated with increased pain sensitivity and others reporting the opposite.[68,69,133] Due to the large sample sizes in our studies, we could control for confounding in the analyses more extensively than has been possible in previous case-control studies.

Some researchers have found that hyperalgesia in IBS is found mainly in the lower body parts and is to a lesser degree present in the upper body.[69,71,85] This finding suggests altered pain modulation at peripheral and spinal levels rather than at supraspinal levels in the central nervous system. We found that IBS was independently associated with widespread increased pain sensitivity, after controlling for comorbid non-abdominal pain, with all pain tests conducted at sites that are remote from the location of clinical pain (finger, arm, and shoulder). Therefore, our results support theories of central pain sensitization mechanisms in IBS, at least at higher spinal or supraspinal levels. It is important to emphasize that our findings do not rule out the possibility that topographical pain sensitivity differences in IBS occur in addition to the widespread hyperaglesia documented in our studies.

Our findings are further strengthened by the dose-dependent relationship between degree of abdominal pain in IBS and degree of hyperalgesia, a finding that was also significant after adjusting for comorbid pain. A small number of previous studies have reported similar dose-dependent findings.[70,136] Furthermore, our results harmonize with previous *visceral* sensitivity studies among patients with IBS that described a dose-dependent relationship between degree of IBS symptoms and visceral hyperalgesia.[65,137]

The IBS diagnostic criteria require the presence of *pain or discomfort*, and thus the presence of pain is not mandatory. Consequently, 16% of adults but none of the adolescents met the IBS criteria while reporting no abdominal pain. Interestingly, our results suggest an absence of hyperalgesia among these cases. In view of what appears to be qualitative differences between IBS cases reporting and not reporting pain, one might question whether the diagnostic criteria are too inclusive and whether some degree of pain should be mandatory in the diagnostic criteria.

There is considerable controversy regarding the importance of psychological factors in the association between IBS and hyperalgesia. Several studies have found that negative affect is

associated with increased pain sensitivity.[138] These symptoms are common among patients with IBS and could therefore explain why patients with IBS are more pain sensitive than healthy controls (i.e., confounding). Some researchers report that the association between IBS and widespread hyperalgesia is independent of negative affect, whereas others report the opposite.[67,69-71,97,133] Conflicting findings are also described in studies of visceral pain sensitivity in IBS.[65,66,137,139,140] In addition to anxiety and depression, symptoms of somatization have been reported to be associated with hyperalgesia in IBS, at least in patients with IBS with fibromyalgia.[133] Arguments against the latter are discussed in a study by Verne et al. , where somatization and hypervigilance were not found to be associated with increased pain sensitivity in IBS.[71] Overall, the diverging results from previous case-control studies are difficult to interpret. Differences in samples and methodological aspects, such as diverging measurements of comorbid psychiatric symptoms, complicate comparisons. Furthermore, several of the previous studies are limited by small sample sizes, which make them statistically vulnerable for type 2 errors. We found that IBS symptoms were independently associated with increased pain sensitivity among adults after controlling for psychological distress (HSCL > 1.85). The large sample sizes suggest that previous studies that have failed to find an independent association are false negative results. Similar findings were made among adolescents with IBS for heat pain threshold but not for pressure pain threshold after adjusting for psychological distress. In paper 2 in this dissertation, the latter is discussed as a possible type II error due to the relatively small adolescent IBS sample. It should also be noted that we found a convincing trend of lower pain thresholds for all pain modalities, in addition to a lower total threshold sum score, in IBS.

Only a few small studies have examined somatic pain sensitivity in pediatric abdominal pain patients, and only a single study examined pediatric patients with IBS. No difference between children with recurrent abdominal pain or IBS and controls was found in two of the studies that examined pain tolerance.[77,141] In line with our findings, two studies demonstrated lower pain thresholds in children with abdominal pain than in controls.[75,76] However, Zohsel et al. found no pain threshold differences in children with abdominal pain versus controls and argues against generalized hyperalgesia in RAP patients.[78] The pediatric pain sensitivity studies cited here are not only limited by relative small sample sizes but also to a large extent by the lack of measures of comorbid conditions. The importance of both

comorbid pain and psychological distress symptoms has therefore not been assessed in earlier studies of pain sensitivity in pediatric abdominal pain patients, with the exception of one study describing no difference in social and neurotic characteristics between RAP children and no-pain patients.[75] Our study is the first not only to find an association between adolescent IBS and increased pain sensitivity but also, and importantly, to find that this association is independent of comorbid pain and psychological distress. Furthermore, our results are supported by the dose-dependent relationship between degree of abdominal pain in IBS and reduction of the pain thresholds.

To our knowledge, a possible age-dependent difference in pain sensitivity in IBS is poorly described in the literature. In our studies the difference in heat pain thresholds between IBS and controls was comparable for adults and adolescents (- 0.5°C and - 0.8 °C in adult and adolescent IBS vs. controls). Similar trends towards lower pressure pain thresholds in IBS were found in the two age cohorts, but the results are not directly comparable due to different methodology (pain thresholds versus supra-thresholds in adolescents and adults, respectively). The pressure pain threshold differences were small and not significantly lower in adult IBS than in controls. Our results therefore indicate that IBS hyperalgesia, with lower pain thresholds compared to controls, is independent of age. On the other hand, the same age pattern was not found in the cold-pressor pain *tolerance* test. Adult IBS cases had significant lower tolerance than controls, which was also found to be dose-dependently related to degree abdominal pain in IBS. The same difference was not demonstrated in the cold-pressor test among the adolescents, but comparisons are somewhat difficult due to the ten-fold difference in sample size. Still, the lack of pain tolerance differences between adolescent IBS and controls in the additional test (heat and pressure pain) supports the validity of the cold-pressor results. A comparison of our two samples, therefore, indicates that pain tolerance in IBS is age dependent. The reasons for this are not clear. The proportion of subjects with comorbid chronic pain and psychological distress were similar for adult and adolescent IBS cases compared to controls (approximately two and three times higher than among controls, respectively), making it unlikely that these comorbidities contribute to age-related differences. One possibility is that reduced pain tolerance develops as a function of pain duration, which is much longer among adults than

adolescents. Consequently, but theoretically, adults with IBS are more pain sensitized over time. However, this hypothesis will need to be confirmed in prospective studies.

5.3. IBS prevalence and comorbidity

The adult IBS prevalence of 5.3% is in the lower range of the prevalence reported by previous epidemiological reports.[142] It is also lower than Vandvik et al. found in a cross-sectional population-based study in the Hedemark and Oppland Counties of Norway in 2001 (8.4%).[37] Both studies used the IBS Rome II criteria, but the higher prevalence found by Vandvik et al. might be explained by the inclusion of younger adults than in our study (older than 20 vs. older than 30 years of age). Our finding of higher prevalence among adolescents from the same municipality of Tromsø (8.2%) is a further indication that this may be the case, but the IBS criteria differ somewhat in the two studies. The sex and age distribution in the study samples is an important issue when evaluating IBS prevalence, with the knowledge that prevalence is higher in women than in men[32] and decreases with increasing age.[32,37]

When comparing IBS prevalence studies is it important to be aware that the IBS criteria have changed over time, mainly with respect to IBS symptom duration and frequency. The differences are relatively small within the Rome IBS criteria,[143] while poorer agreement is to be expected with the earlier classification criteria (Manning criteria, which are less restrictive compared to the Rome criteria).[144] In a previous Tromsø Study from 1979-80 the prevalence of irritable colon was 8% in men and 13% in women,[145] but these results are not directly comparable with the IBS prevalence estimates in our study due to differences in irritable colon and IBS definitions.

There are few population-based pediatric studies of IBS prevalence, but it is reported to increase during childhood and adolescence.[33] Our estimates were lower than the prevalence found in one North American study in 1996 (17%)[33] but somewhat higher than in a more recent study of younger children in Germany aged 6 to 10 years (4.9 %).[146] The prevalence of IBS is reported to be higher in both a Chinese (19.9%) and Japanese (19%) population-based adolescent study, using the Rome III and II criteria, respectively.[147,148] Again, comparing results is difficult due to differences in the IBS criteria and age composition of the samples. It is possible that the higher prevalence found in the two Asian studies may reflect true cultural and/or ethnic differences, but caveats above make this conclusion uncertain.[149]

The high prevalence of psychological distress (HSCL ≥ 1.85) in adult IBS accords with what is described in the medical literature,[10,37,150] as is the high rate of comorbid chronic pain.[41] Although there are fewer studies on these issues in children and adolescents with IBS, the same pattern of comorbidity has been found in children.[8,44,151] In a study by Gulewitsch et al., IBS symptoms were associated with increased emotional problems compared to children without abdominal pain.[146] These children also had increased levels of somatic symptoms other than IBS, including non-abdominal pain, in accordance with our findings. To our knowledge, only one community-based study of IBS in adolescents has been conducted previously. In that study, a higher frequency of anxiety and depression symptoms were reported in both children and adolescents with IBS compared to controls.[33] A higher prevalence of headache was also reported in that study among children and adolescents with abdominal pain compared to children without, but the prevalence of headache was not specifically reported for the IBS subgroup. Consequently, we have shown that IBS symptoms are common among adolescents in the general population and that these individuals have an overall greater symptom burden, including higher rates of comorbid pain, anxiety, and depression.

5.4. Abdominal pain symptom dimensions and depression

Although there are many studies on comorbid psychological problems in children with abdominal pain,[44,45,146] there is little knowledge about the association of the specific abdominal pain symptom dimension with depressive symptoms. Both IBS and recurrent abdominal pain symptoms in general were associated with symptoms of depression among the adolescents, as described in paper 3 in this dissertation. These findings are in accordance with what is described in previous comparable adult and pediatric studies. As described in paper 3, the association was strong and only slightly attenuated after controlling for sex, parental level of education, and comorbid chronic pain.

To study how specific pain and bowel symptoms were associated with depression, we conducted further investigations within the group of adolescents with recurrent abdominal pain (n = 257). This was done because these symptoms were not measured among pain-free individuals, rendering analysis across this group meaningless. In the subgroup analyses, abdominal pain intensity, distribution (number of pain sites), and comorbid chronic pain remained the only significant predictors of depression. Remaining pain dimensions, including

pain duration and frequency, and bowel symptoms that are mandatory for the IBS diagnosis were not significantly associated with depression in the multi-variable analyses. That abdominal pain per se is important in this association, rather than the associated bowel symptoms in IBS, is further strengthened by the group analyses within the whole study sample, which found comparable odds of depression for IBS (OR 2.4) and abdominal pain (OR = 2.5) compared to controls. To our knowledge there are no similar reports for children or adolescents with IBS or abdominal pain, when studying the specific pain dimensions in multi-variable models, but some reports have described associations between mental health and some of these dimensions individually.[33,42,43,45,152] To our knowledge, the association between abdominal pain and depression, the strongest association in our model, has not been examined previously. Individuals that reported widespread abdominal pain had 5.5 times higher odds of depression compared to pain in a limited abdominal area (95% CI = 2.6-11.8). Furthermore, comorbid chronic pain was found to increase the odds of depression in the same multiple logistic regression analysis (OR = 3.3 with 95% CI = 1.6-6.8). Hence, the pain distribution within and beyond the abdominal region appears to be the strongest predictor of depression. Theoretically, a clinical implication could be that multi-site pain is less "manageable" than single site pain. This implies that depression is a consequence of chronic pain, but it is a question that remains unclear. But this interpretation is supported by results from a prospective study where the number chronic pain sites were found to predict mental stress symptoms later in life in a dose-dependent manner.[49]

The association between abdominal pain severity and mental health problems has been described in a few adult IBS studies. Heitkemper et al. found that psychological distress in IBS is more strongly related to the abdominal pain severity than the predominant stool pattern.[153] Less is known about this association among children and adolescents with IBS, but Hyams et al. found that abdominal pain severity was correlated with both anxiety and depression among adolescents with IBS symptoms.[33]

IBS severity and the burden of the disorder was reported by Bond et al. to be associated with a combination of the different abdominal and bowel symptoms and to the extra-intestinal somatic symptoms in IBS.[154] In that same study, IBS severity was the most highly correlated with the non-bowel symptoms, including abdominal pain symptoms. Whether or not the overall burden of illness is associated with depression or other mental health

problems is not addressed in the study. Another study reported that the degree of abdominal pain is associated with impairments of physical functioning in IBS but not with decreased mental functioning.[155]

Clinically and in research, the sub-classification of IBS is primarily based on the bowel symptoms (diarrhea- or constipation-prominent type of IBS, or mixed subgroups) rather than other abdominal symptoms.[9] The high grade of comorbidity is also not part of the diagnostics and sub-classification of IBS, which some researchers have criticized.[156] The heterogeneity of patients with IBS may be at least partly explained by the lack of discrimination between abdominal pain and discomfort in the IBS criteria. Studies have shown that patients with IBS distinguish these two symptom entities.[157] With the knowledge that the abdominal pain severity in IBS has a great impact on both mental health and quality of life,[52,158] it seems reasonable to distinguish non-pain (discomfort) and abdominal pain patients.

5.5. Methodological considerations

5.5.1. Representativeness

With the combination of a randomly sampled study population and a participation rate of 66% in wave 6 of the Tromsø Study, we could anticipate that the study sample was representative for the general adult population above age 30 years in the municipality of Tromsø, Norway. As discussed above, we found a relatively low IBS prevalence, which could have been a consequence of the somewhat higher proportion older adults (median age 58 years) in addition to not including individuals below age 30 years. The participation rate in the adolescent study was very high (92.4%) and included almost all adolescents in the study area that attended the first year of the upper high school, but missing the adolescents that were not enrolled or quit before the recruitment reached the specific school of attendance. The exact number adolescents not attending or dropping out of first year high school before recruitment is unclear. Therefore, IBS prevalence in these cases remains unknown. But importantly, the associations of IBS and pain sensitivity, as the associations of abdominal pain and depression are most likely not affected by possible under or over estimations of abdominal pain and IBS prevalence's in our study.

5.5.2. Misclassification and measurement errors

As described in the Methods section above, the Rome II and III criteria for IBS were used in the adult and adolescent study, respectively. Most validation studies of the IBS criteria are on clinical samples, and little is known about how the criteria perform in a population-based setting. The IBS criteria sensitivity is considered moderate to reasonably good, compared to clinical IBS diagnostics (0.7-0.9).[19,22] Poorer specificity is reported in discriminating IBS from organic diseases with similar abdominal and bowel symptoms (about 0.7), but the specificity is reported to be better when typical red flag symptoms of organic disease are absent (0.9).[159] Questions about the presence of red flag symptoms were not included in our study, increasing the risk of misclassifying organic gastrointestinal diseases as IBS. But most of these diseases are relative uncommon (e.g., celiac disease and inflammatory bowel disease) in the general population compared to clinical populations. Furthermore, it is even less likely that one these diseases is unrecognized or poorly treated (non-remission). Moreover, having an organic bowel disease does not rule out the possibility of simultaneous comorbid IBS. We therefore believe that the classification of the IBS cases in our study is reasonable correct, with a relative small proportion of cases having an organic disease that causes their abdominal pain and bowel symptoms.

The HSCL (10-item version) was used to identify participants with psychological distress (anxiety and depression symptoms) in papers 1 and 2, and the SMFQ was used in paper 3 to identify individuals with depression. Further details on these questionnaires are described in the Methods section above. These measurements are developed for screening and are used in epidemiological research, but they are not clinical diagnostic tools. As for IBS, there is a risk of misclassification, but is reasonable to anticipate equal distribution of misclassification in IBS and abdominal pain cases as in controls.

The within-session reliability of experimental pain assessment is typically found to be high (Cronbach's alpha > 0.9). [160] Information on the long term stability of these measures (i.e. to what extent they reflect a stable trait of the individual) is more sparse, but there is at least some support for stability over time. [161,162]

Pain sensitivity assessments are still vulnerable for both procedural and technical errors. One of the most important issues was to secure uniform pain sensitivity testing through

standardized procedures, including exactly the same instructions to each participant. Possible technician-related procedural and technical errors would most likely have a random distribution among both the IBS cases and controls and would therefore not affect our study results.

As described in the study flow charts above, participants with comprehension difficulties (e.g., dementia) were excluded in screening before testing and further tests were of the technicians reported as invalid due to non-comprehension. Still, we cannot exclude less evident errors related to how the participants understood the procedural instructions. But we believe that this problem was infrequent and, again, that the distribution of possible errors is equal in both the IBS and control group.

5.5.3. Confounding

Both comorbid pain and psychological distress symptoms are known to be associated with both IBS and pain sensitivity. If and to what degree the same symptoms are confounders in the association between IBS and increased pain sensitivity is unclear, however, mostly due to methodological issues and small sample sizes in previous case-control studies. That we found an independent association between IBS and increased pain sensitivity after adjustments for comorbid pain and psychological distress does not exclude confounding effects. But, as discussed in the papers in this dissertation, the magnitude of IBS as an independent predictor was only slightly attenuated after the same adjustments, indicating only a small if overall present confounding effect.

Unnecessary adjustments could also be a consequence when a co-factor is added in the analyses, but not fulfilling the confounding factor requirements as mentioned above. If the factor is an intermediate factor rather than an independent factor, there is a risk of over-adjustment errors in the analyses. Likewise if the factor is a consequence rather than a cause of the outcome in question. Adjustment errors could also be a consequence in situations where the factor is completely out of the system of interest or only associated with either the main predictor or outcome. In these situations, adjustments will not necessarily alter the magnitude of the crude association but will primarily reduce the precision in the analyses (wider confidence interval). As discussed in paper 2 in this dissertation, psychological distress symptoms were not a significant covariate in the analyses of the pressure pain

thresholds in IBS and controls. Furthermore, the mean difference between IBS and controls was basically unchanged after controlling for psychological distress symptoms, but the confidence interval increased, and the differences between IBS and controls turned out to be non-significant after the same adjustments. This may therefore be an over-adjustment rather than a true confounding effect. As discussed in paper 2, an additional cause may be related to the relatively small IBS sample in the adolescent study (type II error). The same power problem could also explain the somewhat inconsistent heat pain threshold findings in the adult IBS pain subgroups described in paper 1.

Finally, the independent association between IBS and increased pain sensitivity found does not rule out the possibility of residual confounding. In our analyses, as discussed in the papers, symptoms of somatization in IBS are a possible residual confounder.

5.5.4. Incomplete data

If there were missing data in the IBS module and participants had responded in the prior question that they had no abdominal pain during the last year, they were categorized as no-IBS (= controls). But when missing answers in both the IBS module and the prior questionnaire item on chronic abdominal pain they were handled as missing (n = 1621 and 12.5%). The relatively high proportion of incomplete IBS data may have affected the IBS prevalence estimates and, to a lesser degree, the main analyses of associations between IBS and pain sensitivity. Missing answers were somewhat more frequent among both male and the older participants, but we did not discover any evident differences between the two groups in prevalence of comorbid chronic pain and psychological distress.

Imputation techniques were not conducted in our studies, but as described above, missing HSCL scores were replaced by the mean of the completed answers, which is in accordance with procedures followed in other previous studies.[126] Participants missing more than three of 10 answers were handled as missing (n = 684 and 5.3%).

Participants missing physical pain sensitivity measurements were compared with the participants that had undergone the testing. This is described in paper 1, with the finding that there were no differences in IBS prevalence between these two groups, but the participants with missing measurements were somewhat older, more often women, and had

more psychological distress than the participated that had the testing. As described in the paper, these differences were not significant after controlling for sex and age.

5.6. Conclusions, implications, and future studies

The association between IBS and increased widespread pain sensitivity among adolescent and adult individuals in the general population, which was found to be independent of comorbid pain and psychological distress, is the main and most important finding of our project. This finding confirms results from previous clinical case-control studies and expands on previous studies by providing grounds for generalizing to the general population. Our finding provides strong evidence of CNS involvement in the disorder and central pain sensitization mechanisms in IBS.

Furthermore, we found that diffuse widespread abdominal pain, multi-site chronic pain, and abdominal pain intensity were all independently associated with depression. Whether or not the same pain dimensions are risk factors for or consequences of depression remains to be elucidated in prospective studies, but our results support screening for depression among adolescent patients with severe abdominal pain and multi-site pain.

The strong association between abdominal pain intensity and both hyperalgesia and depression support greater attention on abdominal pain *per se* in IBS. By contrast, pain-free IBS and IBS related bowel symptoms were not independently related to these outcomes. This suggests that the diagnostic classification of the disorder may be improved by distinguishing IBS patients with abdominal pain from those reporting discomfort without pain.

Our study was cross-sectional and therefore unsuited for demonstrating order effects and testing hypotheses about causal relationships. The upcoming seventh Tromsø study, where all participants in both the adolescent and adult samples will be invited for a repeated examination, may contribute in better understanding of the temporal relationships between IBS, hyperalgesia and depression.

6. REFERENCES

1. Merskey H. The taxonomy of pain. *The Medical clinics of North America.* Jan 2007;91(1):13-20, vii.
2. Stanton TR, Latimer J, Maher CG, Hancock MJ. How do we define the condition 'recurrent low back pain'? A systematic review. *Eur Spine J.* Apr 2010;19(4):533-539.
3. Stovner LJ. Headache epidemiology: how and why? *The journal of headache and pain.* Jun 2006;7(3):141-144.
4. Phillips K, Clauw DJ. Central pain mechanisms in the rheumatic diseases: future directions. *Arthritis and rheumatism.* Feb 2013;65(2):291-302.
5. Thompson WG. The road to rome. *Gastroenterology.* Apr 2006;130(5):1552-1556.
6. Manning AP, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel. *British medical journal.* Sep 2 1978;2(6138):653-654.
7. Apley J, Naish N. Recurrent abdominal pains: a field survey of 1,000 school children. *Arch Dis Child.* Apr 1958;33(168):165-170.
8. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal pain in Western countries: a systematic review. *Am.J.Gastroenterol.* 2005;100(8):1868-1875.
9. Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology.* Apr 2006;130(5):1377-1390.
10. Cole JA, Rothman KJ, Cabral HJ, Zhang Y, Farraye FA. Migraine, fibromyalgia, and depression among people with IBS: a prevalence study. *BMC gastroenterology.* 2006;6:26.
11. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. *Aliment Pharmacol Ther.* Mar 1 2003;17(5):643-650.
12. Gunnarsson J, Simren M. Peripheral factors in the pathophysiology of irritable bowel syndrome. *Dig.Liver Dis.* 2009;41(11):788-793.
13. Elsenbruch S. Abdominal pain in Irritable Bowel Syndrome: a review of putative psychological, neural and neuro-immune mechanisms. *Brain Behav.Immun.* 2011;25(3):386-394.
14. Mayer EA, Tillisch K. The brain-gut axis in abdominal pain syndromes. *Annual review of medicine.* 2011;62:381-396.
15. Camilleri M. Peripheral mechanisms in irritable bowel syndrome. *The New England journal of medicine.* Oct 25 2012;367(17):1626-1635.
16. Camilleri M, Di Lorenzo C. Brain-gut axis: from basic understanding to treatment of IBS and related disorders. *Journal of pediatric gastroenterology and nutrition.* Apr 2012;54(4):446-453.
17. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology.* Apr 2006;130(5):1527-1537.
18. Vanner SJ, Depew WT, Paterson WG, DaCosta LR, Groll AG, Simon JB, Djurfeldt M. Predictive value of the Rome criteria for diagnosing the irritable bowel syndrome. *Am J Gastroenterol.* Oct 1999;94(10):2912-2917.
19. Ford AC, Bercik P, Morgan DG, Bolino C, Pintos-Sanchez MI, Moayyedi P. Validation of the Rome III Criteria for the Diagnosis of Irritable Bowel Syndrome in Secondary Care. *Gastroenterology.* Aug 28 2013.
20. Helgeland H, Flagstad G, Grotta J, Vandvik PO, Kristensen H, Markestad T. Diagnosing pediatric functional abdominal pain in children (4-15 years old) according to the Rome III Criteria: results from a Norwegian prospective study. *Journal of pediatric gastroenterology and nutrition.* Sep 2009;49(3):309-315.

21. Caplan A, Walker L, Rasquin A. Validation of the pediatric Rome II criteria for functional gastrointestinal disorders using the questionnaire on pediatric gastrointestinal symptoms. *J.Pediatr.Gastroenterol.Nutr.* 2005;41(3):305-316.
22. Engsbro AL, Begtrup LM, Kjeldsen J, Larsen PV, de Muckadell OS, Jarbol DE, Bytzer P. Patients suspected of irritable bowel syndrome--cross-sectional study exploring the sensitivity of Rome III criteria in primary care. *Am J Gastroenterol.* Jun 2013;108(6):972-980.
23. Vandvik PO, Aabakken L, Farup PG. Diagnosing irritable bowel syndrome: poor agreement between general practitioners and the Rome II criteria. *Scandinavian journal of gastroenterology.* May 2004;39(5):448-453.
24. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut.* Sep 1999;45 Suppl 2:Ii43-47.
25. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology.* Apr 2006;130(5):1480-1491.
26. King S, Chambers CT, Huguet A, MacNevin RC, McGrath PJ, Parker L, MacDonald AJ. The epidemiology of chronic pain in children and adolescents revisited: a systematic review. *Pain.* Dec 2011;152(12):2729-2738.
27. Leadley RM, Armstrong N, Lee YC, Allen A, Kleijnen J. Chronic diseases in the European Union: the prevalence and health cost implications of chronic pain. *Journal of pain & palliative care pharmacotherapy.* Dec 2012;26(4):310-325.
28. van Hecke O, Torrance N, Smith BH. Chronic pain epidemiology and its clinical relevance. *British journal of anaesthesia.* Jul 2013;111(1):13-18.
29. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *European journal of pain (London, England).* May 2006;10(4):287-333.
30. Reid KJ, Harker J, Bala MM, Truyers C, Kellen E, Bekkering GE, Kleijnen J. Epidemiology of chronic non-cancer pain in Europe: narrative review of prevalence, pain treatments and pain impact. *Current medical research and opinion.* Feb 2011;27(2):449-462.
31. Grundmann O, Yoon SL. Irritable bowel syndrome: epidemiology, diagnosis and treatment: an update for health-care practitioners. *J Gastroenterol Hepatol.* Apr 2010;25(4):691-699.
32. Quigley EM, Abdel-Hamid H, Barbara G, Bhatia SJ, Boeckxstaens G, De Giorgio R, Delvaux M, Drossman DA, Foxx-Orenstein AE, Guarner F, Gwee KA, Harris LA, Hungin AP, Hunt RH, Kellow JE, Khalif IL, Kruis W, Lindberg G, Olano C, Moraes-Filho JP, Schiller LR, Schmulson M, Simren M, Tzeuton C. A global perspective on irritable bowel syndrome: a consensus statement of the World Gastroenterology Organisation Summit Task Force on irritable bowel syndrome. *Journal of clinical gastroenterology.* May-Jun 2012;46(5):356-366.
33. Hyams JS, Burke G, Davis PM, Rzepski B, Andrulonis PA. Abdominal pain and irritable bowel syndrome in adolescents: a community-based study. *The Journal of pediatrics.* Aug 1996;129(2):220-226.
34. Chiou E, Nurko S. Functional abdominal pain and irritable bowel syndrome in children and adolescents. *Therapy.* May 1 2011;8(3):315-331.
35. Ehlin AG, Montgomery SM, Ekbom A, Pounder RE, Wakefield AJ. Prevalence of gastrointestinal diseases in two British national birth cohorts. *Gut.* Aug 2003;52(8):1117-1121.
36. Yale SH, Musana AK, Kieke A, Hayes J, Glurich I, Chyou PH. Applying case definition criteria to irritable bowel syndrome. *Clinical medicine & research.* May 2008;6(1):9-16.
37. Vandvik PO, Lydersen S, Farup PG. Prevalence, comorbidity and impact of irritable bowel syndrome in Norway. *Scandinavian journal of gastroenterology.* Jun 2006;41(6):650-656.
38. Zhou H, Li D, Cheng G, Fan J, Lu H. An epidemiologic study of irritable bowel syndrome in adolescents and children in South China: a school-based study. *Child Care Health Dev.* 2010;36(6):781-786.

39. Rajindrajith S, Devanarayana NM. Subtypes and Symptomatology of Irritable Bowel Syndrome in Children and Adolescents: A School-based Survey Using Rome III Criteria. *Journal of neurogastroenterology and motility.* Jul 2012;18(3):298-304.
40. Riedl A, Schmidtmann M, Stengel A, Goebel M, Wisser AS, Klapp BF, Monnikes H. Somatic comorbidities of irritable bowel syndrome: a systematic analysis. *Journal of psychosomatic research.* Jun 2008;64(6):573-582.
41. Whitehead WE, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? *Gastroenterology.* Apr 2002;122(4):1140-1156.
42. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic abdominal pain and depressive symptoms: analysis of the national longitudinal study of adolescent health. *Clin.Gastroenterol.Hepatol.* 2008;6(3):329-332.
43. Saps M, Seshadri R, Sztainberg M, Schaffer G, Marshall BM, Di Lorenzo C. A prospective school-based study of abdominal pain and other common somatic complaints in children. *The Journal of pediatrics.* Mar 2009;154(3):322-326.
44. van der Veek SM, Derkx HH, de Haan E, Benninga MA, Boer F. Abdominal pain in Dutch schoolchildren: relations with physical and psychological comorbid complaints in children and their parents. *Journal of pediatric gastroenterology and nutrition.* Oct 2010;51(4):481-487.
45. Vila M, Kramer T, Obiols JE, Garralda ME. Abdominal pain in British young people: associations, impairment and health care use. *Journal of psychosomatic research.* Dec 2012;73(6):437-442.
46. Bouchoucha M, Hejnar M, Devroede G, Babba T, Bon C, Benamouzig R. Anxiety and depression as markers of multiplicity of sites of functional gastrointestinal disorders: A gender issue? *Clinics and research in hepatology and gastroenterology.* Dec 24 2012.
47. Addolorato G, Mirijello A, D'Angelo C, Leggio L, Ferrulli A, Abenavoli L, Vonghia L, Cardone S, Leso V, Cossari A, Capristo E, Gasbarrini G. State and trait anxiety and depression in patients affected by gastrointestinal diseases: psychometric evaluation of 1641 patients referred to an internal medicine outpatient setting. *International journal of clinical practice.* Jul 2008;62(7):1063-1069.
48. Bohman H, Jonsson U, Paaren A, von Knorring L, Olsson G, von Knorring AL. Prognostic significance of functional somatic symptoms in adolescence: a 15-year community-based follow-up study of adolescents with depression compared with healthy peers. *BMC psychiatry.* 2012;12:90.
49. Lien L, Green K, Thoresen M, Bjertness E. Pain complaints as risk factor for mental distress: a three-year follow-up study. *European child & adolescent psychiatry.* Oct 2011;20(10):509-516.
50. Roth-Isigkeit A, Thyen U, Stöven H, Schwarzenberger J, Schmucker P. Pain Among Children and Adolescents: Restrictions in Daily Living and Triggering Factors. *Pediatrics.* February 1, 2005 2005;115(2):e152-e162.
51. Varni JW, Lane MM, Burwinkle TM, Fontaine EN, Youssef NN, Schwimmer JB, Pardee PE, Pohl JF, Easley DJ. Health-related quality of life in pediatric patients with irritable bowel syndrome: a comparative analysis. *J.Dev.Behav.Pediatr.* 2006;27(6):451-458.
52. Rey E, Garcia-Alonso MO, Moreno-Ortega M, Alvarez-Sanchez A, Diaz-Rubio M. Determinants of quality of life in irritable bowel syndrome. *Journal of clinical gastroenterology.* Oct 2008;42(9):1003-1009.
53. Johansson PA, Farup PG, Bracco A, Vandvik PO. How does comorbidity affect cost of health care in patients with irritable bowel syndrome? A cohort study in general practice. *BMC gastroenterology.* 2010;10:31.
54. Hillila MT, Siivola MT, Farkkila MA. Comorbidity and use of health-care services among irritable bowel syndrome sufferers. *Scandinavian journal of gastroenterology.* Jul 2007;42(7):799-806.

55. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A Diet Low in FODMAPs Reduces Symptoms of Irritable Bowel Syndrome. *Gastroenterology.* Sep 24 2013.
56. Simren M, Barbara G, Flint HJ, Spiegel BM, Spiller RC, Vanner S, Verdu EF, Whorwell PJ, Zoetendal EG. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut.* Jan 2013;62(1):159-176.
57. Saps M, Pensabene L, Di Martino L, Staiano A, Wechsler J, Zheng X, Di Lorenzo C. Post-infectious functional gastrointestinal disorders in children. *The Journal of pediatrics.* Jun 2008;152(6):812-816, 816 e811.
58. Marshall JK, Thabane M, Garg AX, Clark WF, Moayyedi P, Collins SM, Investigators WHS. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. *Gut.* May 1, 2010 2010;59(5):605-611.
59. Gwee KA, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, Read NW. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. *Lancet.* Jan 20 1996;347(8995):150-153.
60. Neal KR, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: a six year follow up study. *Gut.* Sep 2002;51(3):410-413.
61. Jones MP, Dilley JB, Drossman D, Crowell MD. Brain-gut connections in functional GI disorders: anatomic and physiologic relationships. *Neurogastroenterol Motil.* Feb 2006;18(2):91-103.
62. Stasi C, Rosselli M, Bellini M, Laffi G, Milani S. Altered neuro-endocrine-immune pathways in the irritable bowel syndrome: the top-down and the bottom-up model. *Journal of gastroenterology.* Nov 2012;47(11):1177-1185.
63. Tanaka Y, Kanazawa M, Fukudo S, Drossman DA. Biopsychosocial model of irritable bowel syndrome. *J.Neurogastroenterol.Motil.* 2011;17(2):131-139.
64. Bouin M, Plourde V, Boivin M, Riberdy M, Lupien F, Laganiere M, Verrier P, Poitras P. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology.* Jun 2002;122(7):1771-1777.
65. Posserud I, Syrous A, Lindstrom L, Tack J, Abrahamsson H, Simren M. Altered rectal perception in irritable bowel syndrome is associated with symptom severity. *Gastroenterology.* 2007;133(4):1113-1123.
66. Guthrie E, Barlow J, Fernandes L, Ratcliffe J, Read N, Thompson DG, Tomenson B, Creed F. Changes in tolerance to rectal distension correlate with changes in psychological state in patients with severe irritable bowel syndrome. *Psychosom.Med.* 2004;66(4):578-582.
67. Bouin M, Meunier P, Riberdy-Poitras M, Poitras P. Pain hypersensitivity in patients with functional gastrointestinal disorders: a gastrointestinal-specific defect or a general systemic condition? *Dig.Dis.Sci.* 2001;46(11):2542-2548.
68. Caldarella MP, Giamberardino MA, Sacco F, Affaitati G, Milano A, Lerza R, Balatsinou C, Laterza F, Pierdomenico SD, Cuccurullo F, Neri M. Sensitivity disturbances in patients with irritable bowel syndrome and fibromyalgia. *Am.J.Gastroenterol.* 2006;101(12):2782-2789.
69. Moshiree B, Price DD, Robinson ME, Gaible R, Verne GN. Thermal and visceral hypersensitivity in irritable bowel syndrome patients with and without fibromyalgia. *Clin.J.Pain.* 2007;23(4):323-330.
70. Zhou Q, Fillingim RB, Riley JL, III, Verne GN. Ischemic hypersensitivity in irritable bowel syndrome patients. *Pain Med.* 2010;11(11):1619-1627.
71. Verne GN, Robinson ME, Price DD. Hypersensitivity to visceral and cutaneous pain in the irritable bowel syndrome. *Pain.* 2001;93(1):7-14.
72. Di Lorenzo C, Youssef NN, Sigurdsson L, Scharff L, Griffiths J, Wald A. Visceral hyperalgesia in children with functional abdominal pain. *The Journal of pediatrics.* Dec 2001;139(6):838-843.
73. Faure C, Wieckowska A. Somatic referral of visceral sensations and rectal sensory threshold for pain in children with functional gastrointestinal disorders. *The Journal of pediatrics.* Jan 2007;150(1):66-71.

74. Halac U, Noble A, Faure C. Rectal sensory threshold for pain is a diagnostic marker of irritable bowel syndrome and functional abdominal pain in children. *The Journal of pediatrics.* Jan 2010;156(1):60-65 e61.
75. Alfven G. The pressure pain threshold (PPT) of certain muscles in children suffering from recurrent abdominal pain of non-organic origin. An algometric study. *Acta paediatrica (Oslo, Norway : 1992).* May 1993;82(5):481-483.
76. Duarte MA, Goulart EM, Penna FJ. Pressure pain threshold in children with recurrent abdominal pain. *Journal of pediatric gastroenterology and nutrition.* Sep 2000;31(3):280-285.
77. Williams AE, Heitkemper M, Self MM, Czyzewski DI, Shulman RJ. Endogenous inhibition of somatic pain is impaired in girls with irritable bowel syndrome compared with healthy girls. *The journal of pain : official journal of the American Pain Society.* Sep 2013;14(9):921-930.
78. Zohsel K, Hohmeister J, Flor H, Hermann C. Somatic pain sensitivity in children with recurrent abdominal pain. *Am.J.Gastroenterol.* 2008;103(6):1517-1523.
79. Nielsen CS, Staud R, Price DD. Individual differences in pain sensitivity: measurement, causation, and consequences. *The journal of pain : official journal of the American Pain Society.* Mar 2009;10(3):231-237.
80. Fillingim RB. Individual differences in pain responses. *Current rheumatology reports.* Oct 2005;7(5):342-347.
81. Hastie BA, Riley JL, 3rd, Robinson ME, Glover T, Campbell CM, Staud R, Fillingim RB. Cluster analysis of multiple experimental pain modalities. *Pain.* Aug 2005;116(3):227-237.
82. Nussbaum EL, Downes L. Reliability of clinical pressure-pain algometric measurements obtained on consecutive days. *Physical therapy.* Feb 1998;78(2):160-169.
83. Persson AL, Brogardh C, Sjolund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. *Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine.* Jan 2004;36(1):17-27.
84. Soee AB, Thomsen LL, Tornoe B, Skov L. Reliability of four experimental mechanical pain tests in children. *Journal of pain research.* 2013;6:103-110.
85. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain.* Mar 2011;152(3 Suppl):S2-15.
86. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *The journal of pain : official journal of the American Pain Society.* Sep 2009;10(9):895-926.
87. Werner MU, Mjobo HN, Nielsen PR, Rudin A. Prediction of postoperative pain: a systematic review of predictive experimental pain studies. *Anesthesiology.* Jun 2010;112(6):1494-1502.
88. Wilder-Smith CH. The balancing act: endogenous modulation of pain in functional gastrointestinal disorders. *Gut.* Nov 2011;60(11):1589-1599.
89. Zhou Q, Fillingim RB, Riley JL, III, Malarkey WB, Verne GN. Central and peripheral hypersensitivity in the irritable bowel syndrome. *Pain.* 2010;148(3):454-461.
90. Verne GN, Robinson ME, Vase L, Price DD. Reversal of visceral and cutaneous hyperalgesia by local rectal anesthesia in irritable bowel syndrome (IBS) patients. *Pain.* 2003;105(1-2):223-230.
91. Aggarwal A, Cutts TF, Abell TL, Cardoso S, Familoni B, Bremer J, Karas J. Predominant symptoms in irritable bowel syndrome correlate with specific autonomic nervous system abnormalities. *Gastroenterology.* Apr 1994;106(4):945-950.
92. Camilleri M, Ford MJ. Functional gastrointestinal disease and the autonomic nervous system: a way ahead? *Gastroenterology.* Apr 1994;106(4):1114-1118.
93. Kirchner A, Stefan H, Bastian K, Birklein F. Vagus nerve stimulation suppresses pain but has limited effects on neurogenic inflammation in humans. *European journal of pain (London, England).* Jul 2006;10(5):449-455.
94. Lomax AE, Sharkey KA, Furness JB. The participation of the sympathetic innervation of the gastrointestinal tract in disease states. *Neurogastroenterol Motil.* Jan 2010;22(1):7-18.

95. Tache Y, Kiank C, Stengel A. A role for corticotropin-releasing factor in functional gastrointestinal disorders. *Current gastroenterology reports.* Aug 2009;11(4):270-277.
96. Staud R, Cannon RC, Mauderli AP, Robinson ME, Price DD, Vierck CJ, Jr. Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome. *Pain.* Mar 2003;102(1-2):87-95.
97. Piche M, Arsenault M, Poitras P, Rainville P, Bouin M. Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome *Pain.* 2010;148(1):49-58.
98. Tillisch K, Mayer EA, Labus JS. Quantitative Meta-Analysis Identifies Brain Regions Activated During Rectal Distension in Irritable Bowel Syndrome. *Gastroenterology.* 2010.
99. Verne GN, Himes NC, Robinson ME, Gopinath KS, Briggs RW, Crosson B, Price DD. Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. *Pain.* 2003;103(1-2):99-110.
100. Heaton KW, O'Donnell LJ, Braddon FE, Mountford RA, Hughes AO, Cripps PJ. Symptoms of irritable bowel syndrome in a British urban community: consulters and nonconsulters. *Gastroenterology.* Jun 1992;102(6):1962-1967.
101. Simren M, Abrahamsson H, Svedlund J, Bjornsson ES. Quality of life in patients with irritable bowel syndrome seen in referral centers versus primary care: the impact of gender and predominant bowel pattern. *Scandinavian journal of gastroenterology.* May 2001;36(5):545-552.
102. van der Horst HE, van Dulmen AM, Schellevis FG, van Eijk JT, Fennis JF, Bleijenberg G. Do patients with irritable bowel syndrome in primary care really differ from outpatients with irritable bowel syndrome? *Gut.* Nov 1997;41(5):669-674.
103. Ohman L, Simren M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nature reviews. Gastroenterology & hepatology.* Mar 2010;7(3):163-173.
104. Ringel Y, Maharshak N. The Intestinal Microbiota and Immune Function in the Pathogenesis of Irritable Bowel Syndrome. *American journal of physiology. Gastrointestinal and liver physiology.* Jul 25 2013.
105. Gibson PR, Shepherd SJ. Evidence-based dietary management of functional gastrointestinal symptoms: The FODMAP approach. *J Gastroenterol Hepatol.* Feb 2010;25(2):252-258.
106. Wedlake L, A'Hern R, Russell D, Thomas K, Walters JR, Andreyev HJ. Systematic review: the prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther.* Oct 2009;30(7):707-717.
107. Kidd M, Modlin IM, Gustafsson BI, Drozdov I, Hauso O, Pfragner R. Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American journal of physiology. Gastrointestinal and liver physiology.* Aug 2008;295(2):G260-272.
108. Chitkara DK, van Tilburg MA, Blois-Martin N, Whitehead WE. Early life risk factors that contribute to irritable bowel syndrome in adults: a systematic review. *Am J Gastroenterol.* Mar 2008;103(3):765-774; quiz 775.
109. Goodwin L, White PD, Hotopf M, Stansfeld SA, Clark C. Life course study of the etiology of self-reported irritable bowel syndrome in the 1958 British birth cohort. *Psychosomatic medicine.* Feb 2013;75(2):202-210.
110. Nicholl BI, Halder SL, Macfarlane GJ, Thompson DG, O'Brien S, Musleh M, McBeth J. Psychosocial risk markers for new onset irritable bowel syndrome--results of a large prospective population-based study. *Pain.* Jul 2008;137(1):147-155.
111. Drossman DA, Talley NJ, Leserman J, Olden KW, Barreiro MA. Sexual and physical abuse and gastrointestinal illness. Review and recommendations. *Annals of internal medicine.* Nov 15 1995;123(10):782-794.
112. Talley NJ, Fett SL, Zinsmeister AR, Melton LJ, 3rd. Gastrointestinal tract symptoms and self-reported abuse: a population-based study. *Gastroenterology.* Oct 1994;107(4):1040-1049.

113. Ramchandani PG, Stein A, Hotopf M, Wiles NJ. Early parental and child predictors of recurrent abdominal pain at school age: results of a large population-based study. *J.Am.Acad.Child Adolesc.Psychiatry.* 2006;45(6):729-736.
114. Helgeland H, Van Roy B, Sandvik L, Markestad T, Kristensen H. Paediatric functional abdominal pain: significance of child and maternal health. A prospective study. *Acta paediatrica (Oslo, Norway : 1992).* Nov 2011;100(11):1461-1467.
115. Campo JV, Bridge J, Lucas A, Savorelli S, Walker L, Di Lorenzo C, Iyengar S, Brent DA. Physical and emotional health of mothers of youth with functional abdominal pain. *Archives of pediatrics & adolescent medicine.* Feb 2007;161(2):131-137.
116. Kalantar JS, Locke GR, III, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. *Gut.* 2003;52(12):1703-1707.
117. Saito YA, Petersen GM, Larson JJ, Atkinson EJ, Fridley BL, de Andrade M, Locke GR, III, Zimmerman JM, Almazar-Elder AE, Talley NJ. Familial aggregation of irritable bowel syndrome: a family case-control study. *Am.J.Gastroenterol.* 2010;105(4):833-841.
118. Nielsen CS, Knudsen GP, Steingrímsdóttir Ó. Twin studies of pain. *Clinical Genetics.* 2012;82(4):331-340.
119. D'Amato M. Genes and functional GI disorders: from casual to causal relationship. *Neurogastroenterol Motil.* Aug 2013;25(8):638-649.
120. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: The Tromsø Study. *International Journal of Epidemiology.* August 1, 2012 2012;41(4):961-967.
121. Olden KW. Diagnosis of irritable bowel syndrome. *Gastroenterology.* 2002;122.
122. Saito YA, Talley NJ, J. Melton L, Fett S, Zinsmeister AR, Locke GR. The effect of new diagnostic criteria for irritable bowel syndrome on community prevalence estimates. *Neurogastroenterology & Motility.* 2003;15(6):687-694.
123. Sandanger I, Moum T, Ingebrigtsen G, Sorensen T, Dalgard OS, Bruusgaard D. The meaning and significance of caseness: the Hopkins Symptom Checklist-25 and the Composite International Diagnostic Interview. II. *Soc.Psychiatry Psychiatr.Epidemiol.* 1999;34(1):53-59.
124. Sandanger I, Moum T, Ingebrigtsen G, Dalgard OS, Sorensen T, Bruusgaard D. Concordance between symptom screening and diagnostic procedure: the Hopkins Symptom Checklist-25 and the Composite International Diagnostic Interview I. *Soc.Psychiatry Psychiatr.Epidemiol.* 1998;33(7):345-354.
125. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L. The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. *Behav.Sci.* 1974;19(1):1-15.
126. Strand BH, Dalgard OS, Tambs K, Rognerud M. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). *Nord.J.Psychiatry.* 2003;57(2):113-118.
127. Angold A, Costello E, Messer S, Pickles A, Winder F, Silver D. Development of a short questionnaire for use in epidemiological studies of depression in children and adolescents. *International Journal of Methods in Psychiatric Research.* 1995;5:237 - 249.
128. Angold A, Erkanli A, Silberg J, Eaves L, Costello EJ. Depression scale scores in 8-17-year-olds: effects of age and gender. *Journal of child psychology and psychiatry, and allied disciplines.* Nov 2002;43(8):1052-1063.
129. Thapar A, McGuffin P. Validity of the shortened Mood and Feelings Questionnaire in a community sample of children and adolescents: a preliminary research note. *Psychiatry Res.* 1998;81:259 - 268.
130. Price DD, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. *Pain.* Feb 1994;56(2):217-226.
131. Greenspan JD, Slade GD, Bair E, Dubner R, Fillingim RB, Ohrbach R, Knott C, Diatchenko L, Liu Q, Maixner W. Pain sensitivity and autonomic factors associated with development of TMD: the OPPERA prospective cohort study. *The journal of pain : official journal of the American Pain Society.* Dec 2013;14(12 Suppl):T63-74.e61-66.

132. Price DD, Craggs JG, Zhou Q, Verne GN, Perlstein WM, Robinson ME. Widespread hyperalgesia in irritable bowel syndrome is dynamically maintained by tonic visceral impulse input and placebo/nocebo factors: evidence from human psychophysics, animal models, and neuroimaging. *Neuroimage.* 2009;47(3):995-1001.
133. Chang L, Mayer EA, Johnson T, Fitzgerald LZ, Naliboff B. Differences in somatic perception in female patients with irritable bowel syndrome with and without fibromyalgia. *Pain.* 2000;84(2-3):297-307.
134. Wilder-Smith CH, Robert-Yap J. Abnormal endogenous pain modulation and somatic and visceral hypersensitivity in female patients with irritable bowel syndrome. *World journal of gastroenterology : WJG.* Jul 21 2007;13(27):3699-3704.
135. Rodrigues AC, Nicholas VG, Schmidt S, Mauderli AP. Hypersensitivity to cutaneous thermal nociceptive stimuli in irritable bowel syndrome. *Pain.* 2005;115(1-2):5-11.
136. Zhou Q, Fillingim RB, Riley JL, III, Verne GN. Thermal hypersensitivity in a subset of irritable bowel syndrome patients. *World J.Gastroenterol.* 2009;15(26):3254-3260.
137. van der Veek PP, Van Rood YR, Masclee AA. Symptom severity but not psychopathology predicts visceral hypersensitivity in irritable bowel syndrome. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* Mar 2008;6(3):321-328.
138. Lee JE, Watson D, Frey-Law LA. Psychological factors predict local and referred experimental muscle pain: a cluster analysis in healthy adults. *European journal of pain (London, England).* Jul 2013;17(6):903-915.
139. de Medeiros MT, Carvalho AF, Oliveira Lima JW, Dos Santos AA, de Oliveira RB, Nobre ESMA. Impact of depressive symptoms on visceral sensitivity among patients with different subtypes of irritable bowel syndrome. *J.Nerv.Ment.Dis.* 2008;196(9):711-714.
140. Sabate JM, Veyrac M, Mion F, Siproudhis L, Ducrotte P, Zerbib F, Grimaud JC, Dapoigny M, Dyard F, Coffin B. Relationship between rectal sensitivity, symptoms intensity and quality of life in patients with irritable bowel syndrome. *Aliment.Pharmacol.Ther.* 2008;28(4):484-490.
141. Dufton LM, Dunn MJ, Slosky LS, Compas BE. Self-reported and laboratory-based responses to stress in children with recurrent pain and anxiety. *Journal of pediatric psychology.* Jan 2011;36(1):95-105.
142. Saito YA, Schoenfeld P, Locke GR, III. The epidemiology of irritable bowel syndrome in North America: a systematic review. *Am.J.Gastroenterol.* 2002;97(8):1910-1915.
143. Park DW, Lee OY, Shim SG, Jun DW, Lee KN, Kim HY, Lee HL, Yoon BC, Choi HS. The Differences in Prevalence and Sociodemographic Characteristics of Irritable Bowel Syndrome According to Rome II and Rome III. *J.Neurogastroenterol.Motil.* 2010;16(2):186-193.
144. Hillila MT, Farkkila MA. Prevalence of irritable bowel syndrome according to different diagnostic criteria in a non-selected adult population. *Aliment.Pharmacol.Ther.* 2004;20(3):339-345.
145. Johnsen R, Jacobsen BK, Forde OH. Associations between symptoms of irritable colon and psychological and social conditions and lifestyle. *British medical journal (Clinical research ed.).* Jun 21 1986;292(6536):1633-1635.
146. Gulewitsch MD, Enck P, Schwille-Kiuntke J, Weimer K, Schlarb AA. Rome III criteria in parents' hands: pain-related functional gastrointestinal disorders in community children and associations with somatic complaints and mental health. *European journal of gastroenterology & hepatology.* Oct 2013;25(10):1223-1229.
147. Endo Y, Shoji T, Fukudo S, Machida T, Noda S, Hongo M. The features of adolescent irritable bowel syndrome in Japan. *J.Gastroenterol.Hepatol.* 2011;26 Suppl 3:106-109.
148. Zhou H, Yao M, Cheng GY, Chen YP, Li DG. Prevalence and associated factors of functional gastrointestinal disorders and bowel habits in Chinese adolescents: a school-based study. *J.Pediatr.Gastroenterol.Nutr.* 2011;53(2):168-173.
149. Kang JY. Systematic review: the influence of geography and ethnicity in irritable bowel syndrome. *Aliment Pharmacol Ther.* Mar 15 2005;21(6):663-676.

150. Mykletun A, Jacka F, Williams L, Pasco J, Henry M, Nicholson GC, Kotowicz MA, Berk M. Prevalence of mood and anxiety disorder in self reported irritable bowel syndrome (IBS). An epidemiological population based study of women. *BMC gastroenterology.* 2010;10:88.
151. Campo JV, Bridge J, Ehmann M, Altman S, Lucas A, Birmaher B, Di Lorenzo C, Iyengar S, Brent DA. Recurrent abdominal pain, anxiety, and depression in primary care. *Pediatrics.* Apr 2004;113(4):817-824.
152. Campo Jv Fau - Bridge J, Bridge J Fau - Ehmann M, Ehmann M Fau - Altman S, Altman S Fau - Lucas A, Lucas A Fau - Birmaher B, Birmaher B Fau - Di Lorenzo C, Di Lorenzo C Fau - Iyengar S, Iyengar S Fau - Brent DA, Brent DA. Recurrent abdominal pain, anxiety, and depression in primary care. *Pediatrics.* 20040402 DCOM- 20040513 2004;113(1098-4275 (Electronic)):817-824.
153. Heitkemper M, Cain K, Shulman R, Burr R, Poppe A, Jarrett M. Subtypes of Irritable Bowel Syndrome Based on Abdominal Pain/Discomfort Severity and Bowel Pattern. *Digestive Diseases and Sciences.* 2011;56(7):2050-2058.
154. Bond B, Quinlan J, Dukes GE, Mearin F, Clouse RE, Alpers DH. Irritable bowel syndrome: more than abdominal pain and bowel habit abnormalities. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* Jan 2009;7(1):73-79.
155. Koloski NA, Boyce PM, Jones MP, Talley NJ. What level of IBS symptoms drives impairment in health-related quality of life in community subjects with irritable bowel syndrome? Are current IBS symptom thresholds clinically meaningful? *Qual Life Res.* Jun 2012;21(5):829-836.
156. Alpers DH. Multidimensionality of symptom complexes in irritable bowel syndrome and other functional gastrointestinal disorders. *Journal of psychosomatic research.* Jun 2008;64(6):567-572.
157. Spiegel BM, Bolus R, Agarwal N, Sayuk G, Harris LA, Lucak S, Esrailian E, Chey WD, Lembo A, Karsan H, Tillisch K, Talley J, Chang L. Measuring symptoms in the irritable bowel syndrome: development of a framework for clinical trials. *Aliment Pharmacol Ther.* Nov 2010;32(10):1275-1291.
158. Spiegel BM, Gralnek IM, Bolus R, Chang L, Dulai GS, Mayer EA, Naliboff B. Clinical determinants of health-related quality of life in patients with irritable bowel syndrome. *Archives of internal medicine.* Sep 13 2004;164(16):1773-1780.
159. Whitehead WE, Drossman DA. Validation of symptom-based diagnostic criteria for irritable bowel syndrome: a critical review. *Am.J.Gastroenterol.* 2010;105(4):814-820.
160. Nielsen CS, Stubhaug A, Price DD, Vassend O, Czajkowski N, Harris JR. Individual differences in pain sensitivity: genetic and environmental contributions. *Pain.* May 2008;136(1-2):21-29.
161. Rosier EM, Iadarola MJ, Coghill RC. Reproducibility of pain measurement and pain perception. *Pain.* Jul 2002;98(1-2):205-216.
162. Quiton RL, Greenspan JD. Across- and within-session variability of ratings of painful contact heat stimuli. *Pain.* Jul 15 2008;137(2):245-256.