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GENETIC EPIDEMIOLOGICAL STUDIES OF THE FUNCTIONAL SOMATIC SYNDROMES

CHRONIC WIDESPREAD PAIN AND CHRONIC FATIGUE

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To my parents

ABSTRACT

Fibromyalgia and chronic fatigue syndrome are two symptom-defined conditions with many physical symptoms in common, such as muscle pain, fatigue, unrefreshing sleep, and impairment in memory or concentration. These two conditions often co-occur and frequently co-exist with other symptom-defined conditions (e.g., irritable bowel syndrome and recurrent headache), have a female predominance, and share psychosocial or psychiatric characteristics. It has been suggested that fibromyalgia, chronic fatigue syndrome, and other symptom-defined conditions can be considered as syndromes that share underlying pathogenesis, and hence named “functional somatic syndromes”. To date, little is known about the causes of functional somatic syndromes and their co-occurrence (comorbidity). The present study aimed at investigating the etiology of this comorbidity with a focus on chronic widespread pain (the cardinal symptom of fibromyalgia) and chronic fatigue.

Data were obtained from the participants in the Screening Across the Lifespan Twins (SALT) study of the population-based Swedish Twin Registry. All living, contactable, and consenting twins born in Sweden before December 1958 were contacted between March 1998 and December 2002 to participate in a computer-assisted telephone interview. Of 61,355 eligible twins, 44,897 individuals (73.2%) participated in the interview which screened, amongst others, for chronic widespread pain, chronic fatigue, irritable bowel syndrome, recurrent headache, major depression, and generalized anxiety disorder. Zygosity was determined using questions regarding childhood similarity. Psychological risk factors (personality and stress) were assessed using questionnaires administered to monozygotic and same-sex dizygotic twins in 1972-73. Univariate and multivariate twin analyses were implemented in order to estimate the relative importance of genetic and environmental influences on chronic widespread pain, to assess sex differences in the estimates, and to determine the etiological model that best explains the comorbidity of the functional somatic syndromes. Matched case-control and co-twin case-control analyses were performed in order to evaluate the associations between chronic widespread pain and its comorbid conditions, to examine premorbid risks of psychological factors for chronic fatigue, and to assess familial (genetic and family environmental) influences on these associations.

Modest genetic influences and no family environmental influences were found in chronic widespread pain. The estimates did not differ significantly by sex. Strong associations were found between chronic widespread pain and chronic fatigue, followed in magnitude by irritable bowel syndrome. Associations of chronic widespread pain with psychiatric disorders were no longer significant when discordant monozygotic twins were used, whereas associations with most of the other conditions decreased but remained significant, suggesting familial influences on the associations. Emotional instability and perceived stress were significantly associated with chronic fatigue screened ≥ 25 years later in subjects aged < 65 . When monozygotic twins were used, the association with emotional instability was no longer significant but that with perceived stress increased in the severer definition of chronic fatigue, suggesting different effects of genetic influences on the associations. Finally, for women a model with two latent traits shared by four functional somatic syndromes and two psychiatric disorders best explains the etiology of their comorbidities. The psychiatric disorders loaded on only one of the two latent traits, suggesting that the two latent traits reflect the affective and non-affective (or physiological) aspects of functional symptoms. Each illness is also influenced by genetic and environmental factors specific to each. In conclusion, the comorbidity of functional somatic syndromes is attributed to latent etiological traits (one of which is affective and the other not) shared by these syndromes, whereas the differences among them are attributable to genetic and environmental factors specific to each illness.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-IV).

- I. Kato K, Sullivan PF, Evengård B, Pedersen NL
Importance of genetic influences on chronic widespread pain.
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- II. Kato K, Sullivan PF, Evengård B, Pedersen NL
Chronic widespread pain and its comorbidities: a population-based study.
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- III. Kato K, Sullivan PF, Evengård B, Pedersen NL
Premorbid predictors of chronic fatigue.
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- IV. Kato K, Sullivan PF, Evengård B, Pedersen NL
A population-based twin study of functional somatic syndromes.
Submitted for publication

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LIST OF ABBREVIATIONS

A	additive genetic factor
Ac	common additive genetic factor
ACR	The American College of Rheumatology
ACTH	adrenocorticotrophic hormone
AIC	Akaike Information Criterion
As	specific additive genetic factor
BMI	body mass index
C	shared family environmental factor
CDC	The United States Centers for Disease Control and Prevention
CFS	chronic fatigue syndrome
CI	confidence interval
COMT	catecholamine-O-methyltransferase
CRH	corticotropin-releasing hormone
CWP	chronic widespread pain
df	degrees of freedom
<i>DSM-IV</i>	<i>Diagnostic and Statistical Manual of Mental Disorders</i> , 4th edition
DZ	dizygotic twin
E	nonshared environmental factor
Ec	common nonshared environmental factor
Es	specific nonshared environmental factor
fMRI	functional magnetic resonance imaging
GAD	generalized anxiety disorder
GEE	generalized estimating equations
GERD	gastroesophageal reflux disease
HPA	hypothalamic-pituitary-adrenal
IBS	irritable bowel syndrome
L	latent factor
MD	major depression
MZ	monozygotic twin
OR	odds ratio
<i>P</i>	P-value
PET	positron emission tomography
r_a	genetic correlation
r_c	environmental correlation
RH	recurrent headache
SALT	Screening Across the Lifespan Twins
SPECT	single photon emission computed tomography
STR	The Swedish Twin Registry
χ^2	chi-square
-2LL	-2 log-likelihood

1 INTRODUCTION

It is part of daily life to experience subjective changes in one's body. As a human being, no one is free from concerns about such changes (i.e., symptoms), including pain, fatigue, weakness, insomnia, and so on. In most cases, these bodily (or somatic) symptoms are only transient and fully curable by resting, sleeping more, doing light exercises and relaxation, or taking some prescribed or over-the-counter medicines. Usually, those kinds of symptoms seldom become so severe that they impair one's daily life.

However, in some cases, somatic symptoms can become persistent and disabling. What puzzles physicians is that a sizable number of symptoms remain unexplained by objectively identifiable abnormalities (i.e., diseases) even after extensive medical examinations or assessments. These medically unexplained symptoms are sometimes described as "functional". Moreover, these symptoms are often multiple and present concomitantly, and may be called a "functional somatic syndrome". Above all, two such syndromes that are relatively prevalent at clinics, refractory to most treatments and thereby challenging to both clinicians and researchers are fibromyalgia and chronic fatigue syndrome.

A well-established clinical finding is that fibromyalgia and chronic fatigue syndrome are frequently comorbid, that is to say, many patients have the two conditions at the same time. Given the high frequency of co-occurrence of these two and other functional syndromes, it is tempting to speculate that they share pathogenesis to some degree. As yet, little is known about the etiology of each syndrome, and even less about the causes or mechanisms of coexistence. The overall aim of this thesis is to explore the causal nexus of these enigmatic syndromes for understanding and eventually fighting them further.

2 BACKGROUND

2.1 WHAT ARE FUNCTIONAL SOMATIC SYNDROMES?

2.1.1 Definition of the concept

In modern medicine, diagnoses can be given when symptoms are explained by pathologically defined diseases. If the symptoms do not fall in the category of an identifiable disease, physicians will confront difficulties. Such difficulties might leave the patients undiagnosed, lead to ambiguous diagnosis, or even prevent physicians from acknowledging the existence of the symptoms. These unexplained symptoms have been described over the past centuries, and given various names and diagnoses in the absence of demonstrable pathology (Moldofsky et al. 1975). Recently, the term *functional somatic syndrome* has been increasingly used in the literature to refer to a condition in which patients present with unexplained bodily (or somatic) symptoms such as fatigue, muscle pain, or abdominal pain. Functional somatic syndrome can be defined as a set of somatic symptoms that is often multiple and disabling but remains unexplained by identifiable disease (Mayou & Farmer 2002). Although no consensus has been reached yet, the term 'functional somatic syndrome' seems to be useful because this is a purely descriptive label with no etiologic connotations (Feinstein 2001). In fact, functional somatic symptoms or syndromes are commonly found in clinical studies. For example, in a study at primary care clinics in the United Kingdom (Peveler et al. 1997), functional symptoms and syndromes accounted for 20% of all consultations. A cross-cultural study in 14 countries conducted by the World Health Organization (Gureje et al. 1997) found that functional symptoms were common and disabling in primary care patients in all countries and cultures studied.

Among many conditions that are considered as functional syndromes, fibromyalgia and chronic fatigue syndrome are particularly devastating, controversial, and therefore notable. Other conditions with functional symptoms include irritable bowel syndrome, migraine and tension-type headache, myofascial pain and temporomandibular joint syndrome, chronic pelvic pain (in women), multiple chemical sensitivity, and so on. One of the salient features of fibromyalgia and chronic fatigue syndrome is that these two are highly systemic whereas most of the others are organ-specific. As discussed later, it is frequently observed in clinical practice that a number of unexplained symptoms or syndromes share demographic, clinical, and psychosocial characteristics (e.g., Aaron & Buchwald 2001). Such observations have led some researchers to suggest that the characteristics are in common due to underlying mechanisms in common (Wessely et al. 1999). The considerable overlap has also posed issues about the distinctness of each syndrome, particularly fibromyalgia (Wessely & Hotopf 1999). Despite various etiologies proposed with different names, none has been definitive (Feinstein 2001). Throughout the thesis, special focus will be on fibromyalgia or its cardinal feature, chronic widespread pain, and chronic fatigue syndrome.

2.1.2 Fibromyalgia and chronic widespread pain

Fibromyalgia is a chronic condition that is characterized by widespread musculoskeletal pain and tenderness at specific body locations. The American College of Rheumatology (ACR) proposed consensus classification criteria for fibromyalgia (Wolfe et al. 1990). Fibromyalgia is defined as widespread pain lasting

at least 3 months, with pain on palpation at no less than 11 of 18 specified tender points on the body. The diagnosis does not have exclusionary criteria. The ACR definition has relatively high sensitivity and specificity: 88% and 81%, respectively (Wolfe et al. 1990). The term *chronic widespread pain* refers to widespread pain lasting at least 3 months, regardless of a tender point count. The pain is usually perceived as arising from muscle or soft tissue, but joint pain is often reported as well. The stiffness normally feels worse early in the morning, and may be aggravated by increased exertion, soft tissue injuries, lack of sleep, exposure to cold air, and mental stress. Other symptoms that are commonly reported by individuals with fibromyalgia are fatigue, sleep disturbance, mood disturbance, irritable bowel, and headache (Wolfe 1996).

2.1.3 Chronic fatigue syndrome

Chronic fatigue syndrome is characterized by persistent, disabling fatigue unexplained by a conventional medical diagnosis. The 1994 United States Centers for Disease Control and Prevention (CDC) criteria are currently considered the dominating definition of chronic fatigue syndrome (Fukuda et al. 1994). The definition of chronic fatigue syndrome requires at least 6 months of new-onset symptoms of debilitating fatigue accompanied by 4 or more out of 8 specified symptoms (i.e., impaired memory or concentration, sore throat, tender lymph nodes, muscle pain, multiple joint pain, new headaches, unrefreshing sleep, and postexertional malaise). Explanatory medical conditions should be ruled out.

The fatigue is frequently accompanied by several somatic symptoms similar to those in fibromyalgia. Common complaints are swollen or tender lymph nodes, sore throat, headaches, muscle and joint pain, poor concentration, decreased memory, and sleep disturbance (Fukuda et al. 1994). The symptoms sometimes occur after an acute infectious-like illness, an injury, or a period of high stress.

2.2 HOW OFTEN DO THE SYNDROMES OCCUR AND CO-OCCUR?

2.2.1 Epidemiology of the syndromes

Studies using community samples have reported that functional somatic syndromes are relatively common in the general population. The prevalence of chronic widespread pain has been estimated from 4.2% to 12.9% in the UK (Croft et al. 1993), the US (Wolfe et al. 1995b), Canada (White et al. 1999a), Israel (Buskila 2000), and Sweden (Bergman et al. 2001; Lindell et al. 2000). The prevalence of fibromyalgia, which can be regarded as a subgroup of chronic widespread pain, has been reported to range between 0.66% and 3.3% in Denmark (Prescott et al. 1993), the US (Wolfe et al. 1995b), Canada (White et al. 1999a), and Sweden (Lindell et al. 2000). Chronic fatigue syndrome is estimated to be less prevalent. Two studies with community samples reported prevalence of 0.23% and 0.42% in adults (Jason et al. 1999; Reyes et al. 2003). In all, the prevalence was highest in middle age. As discussed below, the vast majority of sufferers are women.

Only very few researchers have studied the incidence and mortality of the syndromes. A Norwegian study (Forseth et al. 1999) following women aged 20-49 for 5.5 years showed that the annual incidence of new cases of fibromyalgia was 583 in 100,000. A difficulty in such studies is that it is unclear whether the increase in incidence is true

or an artifact due to an increased awareness of the syndrome among physicians or patients. In a study with a follow-up of over 8 years in the UK (Macfarlane et al. 2001), the standard mortality rate for chronic widespread pain (defined by their criteria) was estimated as 1.31.

2.2.2 Evidence for comorbidity

In clinical studies, patients with one functional syndrome frequently meet diagnostic criteria for other syndromes. Many studies have reported associations between two of the syndromes such as fibromyalgia and chronic fatigue syndrome (Buchwald & Garrity 1994), fibromyalgia and irritable bowel (Sperber et al. 1999), and fibromyalgia and migraine (Marcus et al. 2005). Aaron et al. (2000) found in a small study at hospital-based clinics that patients with chronic fatigue syndrome had considerable overlaps with fibromyalgia (80%), irritable bowel syndrome (36%), chronic low back pain (32%), and so on. The same authors reviewed the literature and found again a striking proportion of patients with one functional condition meeting criteria for a second condition (Aaron & Buchwald 2001). However, it is known that having two comorbid disorders can increase the likelihood of inclusion in a clinical sample, resulting in biases (Berkson 1946).

An increasing body of literature has examined the co-occurrences of these syndromes in the general population. White et al. (2000) reported that 60% of residents in a Canadian city who were diagnosed as having fibromyalgia also met the criteria for chronic fatigue syndrome. Jason et al. (2000) found in a larger community-based study that 22.7% of individuals with fibromyalgia also met criteria solely for chronic fatigue syndrome. A possible limitation of these two studies is that only a small portion of participants screened actually underwent a detailed medical examination.

2.2.3 Psychiatric comorbidity

Many studies at clinics have reported that patients with these syndromes have relatively high levels of psychiatric symptoms such as anxiety, depression, or other forms of emotional distress. For example, Hudson et al. (1992) reported that patients with fibromyalgia at a tertiary referral center met criteria for lifetime diagnoses of affective and anxiety disorders more frequently than those with rheumatoid arthritis (71% versus 14%, 33% versus 0%, respectively). However, Ahles et al. (1991) reported inconsistent results. At a fatigue clinic, Katon et al. (1991) found that patients with chronic fatigue syndrome had significantly higher prevalence of current and lifetime major depression than those with rheumatoid arthritis. In a Belgian study, Fischler et al. (1997) reported an eight-fold increased risk of generalized anxiety disorder among fatigued patients compared with medical controls.

As was mentioned regarding the comorbidity of the syndromes, investigations based on clinical samples are prone to healthcare seeking biases. Macfarlane et al. (1999) found that in a population-based survey, psychological distress is associated with chronic widespread pain at least partially independent of any effect of healthcare-seeking behavior. Also in a population-based study, Benjamin et al. (2000) found three-fold higher odds of having mood and anxiety disorders in individuals with chronic widespread pain than those without.

The key question is which comes first, or whether pain and other functional symptoms are causes or consequences of psychiatric illness. Hotopf et al. (1998) assessed a population-based birth cohort at two time points (36 and 43 years of age) for somatic symptoms and psychiatric disorder, and found both directions of possible causality, that is, psychiatric symptoms could predict new onset of somatic symptoms and vice versa. The same authors (Hotopf et al. 1999) then reported a relationship of prior experience of physical illness in childhood with the later development of somatization in adulthood. They interpreted the data as reflecting a learned process whereby illness experience leads to symptom monitoring.

Efficacy of cognitive behavioral therapy has been shown to be similar in functional somatic syndromes. In randomized controlled clinical trials, this therapy seems to be beneficial for fibromyalgia (Rossy et al. 1999), chronic fatigue syndrome (Prins et al. 2001), irritable bowel syndrome (Brandt et al. 2002), as well as other chronic pain (Morley et al. 1999). Similar responses to therapy raise the possibility that common underlying mechanisms are shared among the syndromes or between them and psychiatric disorders.

2.3 WHAT IS IN COMMON TO THE SYNDROMES?

2.3.1 Female predominance

Among several characteristics in common to functional somatic syndromes, female predominance is probably the most consistent and striking. Two- to four-fold greater prevalence has also been found in community samples with fibromyalgia (Gran 2003) and chronic fatigue (Evengård et al. 2005) but not significantly greater for irritable bowel syndrome (Saito et al. 2000). It is widely known that psychiatric disorders such as depression are much more common in women (Kessler 2003), although the origins of sex difference are unclear (Fanous et al. 2002). A number of studies therefore examined differences in psychiatric comorbidity between male and female patients with functional somatic syndromes. However, Wolfe et al. (1995a) found no significant sex differences in anxiety and depression among subjects with fibromyalgia. Similar negative results were found in clinical samples with irritable bowel (Corney & Stanton 1990), chronic fatigue syndrome (Buchwald et al. 1994), as well as fibromyalgia (Buskila et al. 2000; Yunus et al. 2004). These findings suggest that the sex differences in the prevalence of functional somatic syndromes may not be due to the female predominance in psychiatric disorders.

2.3.2 Familial influences

Several studies have shown that some of the syndromes appear to 'run in families'. Buskila and colleagues found a higher prevalence of fibromyalgia among offspring of mothers with fibromyalgia and among close blood relatives with fibromyalgia (Buskila et al. 1996; Buskila & Neumann 1997). By comparing first-degree relatives of probands with fibromyalgia and those with rheumatoid arthritis, Arnold et al. (2004) reported an 8.5-fold elevated risk for fibromyalgia in the former. Similarly, Walsh et al. (2001) found a significantly higher prevalence of chronic fatigue syndrome in the relatives of fatigue cases compared with the relatives of medical control subjects. Familial influences have also been suggested in subjects with irritable bowel syndrome (Kalantar et al. 2003; Talley 2005).

Familial aggregation of psychiatric disorders in patients with functional somatic syndromes has also been studied. Hudson et al. (1992) demonstrated that the first-degree relatives of patients with fibromyalgia more frequently met criteria for affective disorders (10%) than did relatives of patients with rheumatoid arthritis (3%). The authors suggested that the high frequency of psychiatric disorders in fibromyalgia patients may represent common pathophysiology (Hudson & Pope 1990). Familial co-aggregation of major depression and fibromyalgia was confirmed in a large clinical study (Arnold et al. 2004) and a large community-based study (Raphael et al. 2004a). By extending their previous hypothesis, Hudson et al. (2003) reported familial co-aggregation of 14 syndromes or disorders, including fibromyalgia, irritable bowel syndrome, migraine, major depression, and generalized anxiety disorder.

However, to run in families does not necessarily mean genetic influences because family members share the same environment some time in their life. To distinguish between genetic and family environmental influences requires genetically informative samples, e.g., twins. In the last two decades, twin studies have been a valuable source of research in a variety of human traits and diseases (Boomsma et al. 2002). Nevertheless, to date, only a few studies have investigated the relative importance of genetic and family environmental influences for functional somatic syndromes. This type of study design is discussed further in Section 2.5.

2.3.3 Stressful experiences

People with functional somatic syndromes often report stressful events, particularly sexual or physical abuse in their childhood, prior to symptom onset. Boisset-Pioro et al. (1995) first showed that female patients in Canada reported a significantly greater frequency of unwanted sexual contact, sexual abuse during childhood, and physical abuse, compared to matched control subjects with other rheumatic disorders. Results in line with their study have been reported by other researchers in the US (Alexander et al. 1998; Taylor et al. 1995; Walker et al. 1997). Taylor & Jason (2001) investigated the frequency of childhood abuse in adults with chronic fatigue syndrome in a community-based study. They found elevated frequencies of childhood sexual and physical abuse in subjects with chronic fatigue syndrome, although this did not reach statistical significance. Recently, Heim et al. (2006) demonstrated three- to eight-fold increased risks for chronic fatigue syndrome across different forms of childhood trauma. Similar relationships have also been found in clinical and community-based studies of individuals with irritable bowel syndrome (Drossman et al. 1990; Talley et al. 1995; Walker et al. 1995). Nevertheless, these studies should be interpreted with caution because of the retrospective design. Raphael et al. (2004b) concluded in their review that the evidence does not support a causal relationship between childhood abuse and chronic pain.

Apart from childhood victimization, a number of studies have found more stress in individuals with functional syndromes than in control subjects. Kivimaki et al. (2004) examined the prospective association between occupational stress and the incidence of newly diagnosed fibromyalgia in hospital workers. Over a period of two years, they found two- to four-fold risks for fibromyalgia in workers with high workload, low decision latitude, and experiences of being bullied at work. However, as Cleare (2004) commented on Kivimaki et al.'s study, the association can be a reflection of a change in illness perception, illness behavior, reverse causation (i.e., undetected pain

symptoms leading to increased stress), and confounding by depression or sleep disturbance.

2.3.4 Personality traits

Certain types of personality are risk factors for physical illnesses. In particular, a personality trait representing negative emotions (e.g., emotional instability, neuroticism, negative affectivity) is consistently associated with a variety of health conditions (Smith & Gallo 2001). Emotional instability is a quantitative personality trait defined as an individual's tendency to experience psychological distress that can be reliably measured by self-report and is relatively stable in an individual over time. Individuals with high scores are characterized by low self-esteem and feelings of anxiety, depression, and guilt. The construct of emotional instability is extraordinarily robust, that is, very similar constructs can be found in essentially every major theory of personality and identifiable across the socioeconomic spectrum and in a diverse range of cultures. A large body of literature demonstrates that emotional instability can predict the morbidity of somatic complaints (Costa & McCrae 1987), hypertension (Spiro et al. 1995), myocardial infarction (Eaker et al. 1992), as well as premature mortality (Somervell et al. 1989). In order to avoid a sense of stigmatization, *emotional instability* is used throughout this thesis without regard to the terminology employed in the original inventories or reports.

It has been known that emotional instability plays a significant role in chronic pain, but not at the level of nociceptive processing (i.e., the reception and transmission of painful or injurious stimuli). In patients with myofascial pain dysfunction, Harkins et al. (1989) found that emotional instability (measured by the Eysenck Personality Inventory; Eysenck & Eysenck 1968) had no influence on the discrimination of thermal pain but appeared to exert powerful influences in the delayed, reflective stage of pain at the level of emotions related to suffering. The authors also examined the effect of extraversion, which had been considered to correlate with higher thresholds or tolerance to pain, but found no influence on temperature pain sensation intensity functions. By comparing subjects with chronic fatigue syndrome, those with multiple sclerosis, and healthy controls, Christodoulou et al. (1999) found elevated levels of Harm Avoidance (a scale of emotionality in Cloninger's Tridimensional Personality Questionnaire; Cloninger 1987) in patients with chronic fatigue syndrome or multiple sclerosis than healthy controls. In another study, however, Wood & Wessely (1999) found no evidence of major differences in personality between patients with chronic fatigue syndrome and those with rheumatoid arthritis.

2.3.5 Neurophysiologic features

The pathophysiological mechanisms in functional somatic syndromes are largely unknown and no single causal agent has been identified. Some features believed to be relevant to the syndromes or symptoms are briefly described in this section.

In fibromyalgia patients, elevated cerebrospinal fluid levels of substance P (Russell et al. 1994), nerve growth factor (Giovengo et al. 1999), nitric oxide metabolites (Larson et al. 2000), and decreased levels of the metabolites of serotonin, norepinephrine, and dopamine (Russell et al. 1992) have been reported. Pall (2001) suggested a pathogenic role of elevated levels of nitric oxide (and its potent oxidant

product, peroxynitrite) for both fibromyalgia and chronic fatigue syndrome. In contrast, elevated levels of substance P were not found in patients with chronic fatigue syndrome (Evengård et al. 1998).

Several neurohormonal perturbations have been found in patients with fibromyalgia, such as low integrated basal cortisol levels and exaggerated adrenocorticotrophic hormone (ACTH) response after exogenous application of corticotropin-releasing hormone (CRH; Crofford et al. 1994). Although these findings suggest down regulation of the hypothalamic-pituitary-adrenal (HPA) axis, it is still unclear how neuroendocrine changes relate to the experience of symptoms, and whether these changes are primary, or secondary to behavioral changes in sleep or exercise (Parker et al. 2001).

Autonomic nervous system dysfunction may also play a role in the pathogenesis of fibromyalgia and chronic fatigue syndrome (Martinez-Lavin & Hermosillo 2000). Torpy et al. (2000) demonstrated that exaggerated norepinephrine release might reflect abnormal regulation of the sympathetic nervous system, possibly secondary to chronically deficient hypothalamic CRH. This might support the notion that fibromyalgia (and related syndromes) represent a primary disorder of the stress system, for example, an inability to respond adequately to stressors resulting in disturbance in stress regulation (Van Houdenhove & Egle 2004).

There is emerging evidence for central sensitization as a key mechanism underlying fibromyalgia, chronic widespread pain, irritable bowel, and migraine or tension-type headache (Yunus 2005). Central sensitization is an increased central neuronal responsiveness and causes hyperalgesia (i.e., exaggerated perception of painful stimuli), allodynia (i.e., a perception of innocuous stimuli as painful), and referred pain. Possible triggers for sensitization are wind-up (i.e., enhanced spinal neuronal responses induced by repetitive noxious stimulation), temporal summation (Li et al. 1999), dysregulated descending inhibitory pathways (Dubner & Ren 1999), and upregulated facilitatory modulation (Zusman 2002). Patients with central sensitization show decreased tolerance not only to pain but also to mechanical pressure, heat, cold, electric stimuli, and so on.

2.4 WHY DO THEY CO-OCCUR?

2.4.1 Hypotheses about underlying mechanisms

Given the high probability of comorbidity and the similarities in characteristic, it has been postulated that these syndromes share etiology in some way. As discussed already, Hudson and colleagues hypothesized that fibromyalgia is a form of a family of disorders, so-called 'affective spectrum disorder' (Hudson & Pope 1990; Hudson et al. 2003). Their interpretation was that the various forms of affective spectrum disorder might share heritable physiologic features. However, as the authors pointed out (Hudson et al. 2004), these studies had several limitations: The samples of probands, control subjects, and their family members were small; information from interviewees and that from non-interviewees were combined; the interviews were not blinded.

In contrast, there are other researchers who have placed more emphasis on neurophysiologic explanations about the comorbidity. Clauw (1995) hypothesized central nervous system hyperactivity as the core of the etiology, which is predisposed

by genetic factors and is responsible for various physical symptoms. In the hypothesis, psychiatric disturbance was considered as either an initiator of the hyperactivity or a modulator of the symptom expression. Similarly, Yunus (2005) proposed 'central sensitivity syndromes', in which central sensitization was put at the center of the illness spectrum. In his review, Yunus criticized the notion of affective spectrum disorder because he thought that depression itself might be the result of central sensitization.

2.4.2 Distinctness of the syndromes

Whether the common mechanism is affective or physiological, findings supporting such mechanisms challenge the distinctness of each functional syndrome. Wessely et al. (1999) argued that the existence of specific syndromes is largely an artifact of medical specialization. By presenting supportive evidence in the literature, the authors went so far as to propose an end to the belief that each "different" syndrome requires its own particular specialist. On the other hand, Barsky & Borus (1999) emphasized the abnormal illness behavior of patients with the belief that they have a serious problem, the sense that their illness is disabling and catastrophic, and the suspicion of physicians' expertise and motivation. The authors also criticized overemphasis on biomedical factors by physicians and sensationalistic media coverage. Some researchers have been even more skeptical and questioned the existence of such syndromes (Ehrlich 2003; Quintner & Cohen 1999).

A few studies have attempted empirical tests for the distinctness of the syndromes. Robbins et al. (1997) interviewed patients at family medicine clinics using a structured instrument that identified the main symptoms of fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome, and somatic manifestations of anxiety and depression. Factor analysis provided a good fit for the model in which the five illnesses were discrete; however, the correlations between the five latent constructs were substantial. These data were re-evaluated in an editorial (Deary 1999), in which principal component analysis identified a higher order common factor responsible for 69% of the variance. Further, in a prospective study at outpatient clinics, Nimnuan et al. (2001) asked patients about 13 functional symptoms and found that a two-factor model better explained the interdependencies among the symptoms.

Despite the considerable overlap, other researchers have opposed the concept of functional somatic syndromes. Researchers who emphasize commonalities among the syndromes are sometimes called 'lumpers', and those who emphasize the distinctness of each syndrome are called 'splitters' (Deary 1999). In a debate by Wessely & White (2004), White concluded that the concept of functional somatic syndromes was unhelpful in understanding illness, etiology, treatment, and outcome. Moss-Morris & Spence (2006) reported supportive results for the distinctions between postinfectious chronic fatigue syndrome and irritable bowel syndrome in a primary care cohort study. Although it is unlikely that only lumpers or splitters are correct, the relative importance of common and unique factors remains controversial.

Thus far, none of the hypotheses is conclusive. This is probably because the etiology of these conditions is more complex and multifactorial than it has been considered. Informative samples that enable researchers to explore the underlying mechanisms are therefore warranted.

2.5 HOW CAN WE EXAMINE THE GENETIC AND ENVIRONMENTAL STRUCTURE?

2.5.1 Usefulness of twin studies in medical science

Twin study applications of quantitative genetics have contributed to medical science to a large extent. The design is a powerful tool particularly when elucidating complex systems with multiple unknown or non-measurable causal factors (Neale & Schmitt 2005). With this technique, it is possible to determine the extent to which genetic and environmental influences are important for individual variation in a trait or an illness. The total variance can be decomposed into genetic and environmental components of variance. The decomposition is based on differences in similarity between monozygotic (or identical) twins, who share all their genes, and dizygotic (or fraternal) twins, who on average share half of their segregating genes. Typically, it is assumed in twin studies that variation in liability to an illness derives from four possible sources: additive genetic variation, nonadditive genetic variation, shared environmental variation (i.e., variation due to the common environment shared by members of the same family), and nonshared environmental variation (i.e., variation due to individuals' unique environments). The last component also includes any variation due to random measurement error in the observation of the phenotype. When data are available only from monozygotic and dizygotic twins reared together, the effects of nonadditive genetic and shared environmental components are confounded and therefore unable to be quantified simultaneously.

Recent progress in multivariate analytic techniques based on structural equation modeling has extended the scope of twin and family studies from estimating heritability for a single variable to analyzing a number of comorbid illnesses or correlated traits at the same time (Boomsma et al. 2002; Posthuma & Boomsma 2005). Further, structural equation modeling can accommodate the analysis of sex differences in heritability estimates through the simultaneous analysis of data from male and female monozygotic and dizygotic twins. By comparing the resemblance between opposite-sex twin pairs and that between same-sex twin pairs, it is possible to test whether different genes influence the same trait in men and women. A growing number of twin registers has been emerging worldwide (Busjahn & Hur 2006).

2.5.2 Twin studies of pain and other somatic symptoms

The twin study design has been used in many studies of chronic pain, fatigue, and other somatic symptoms. MacGregor (2004) reviewed dozens of twin studies on pain-related illnesses, most of which showed evidence for moderate to high heritability. In register-based twin studies, the heritabilities for musculoskeletal diseases such as osteoarthritis, lower back and neck pain, frozen shoulder and tennis elbow, and carpal tunnel syndrome were estimated at 30-46% (Kirk et al. 2002), 35-68% (MacGregor et al. 2004), 40-42% (Hakim et al. 2003), and 46% (Hakim et al. 2002), respectively. The heritability of functional bowel syndrome was estimated at 58% (Morris-Yates et al. 1998) and for headache as over 60% (Larsson et al. 1995). None of these studies detected shared environmental influences for the illnesses. In contrast, significant effects of shared environment were found for pressure pain threshold in adult women (MacGregor et al. 1997), joint pain (Charles et al. 1999), widespread pain in 11-year-old children (Mikkelsen et al. 2001), and chronic fatigue (Buchwald et al. 2001).

Larger register-based twin studies of fatigue showed moderate heritability (43%) with no shared environment (Hickie et al. 1999a), and possible sex differences in genetic (greater in women) and shared environmental (greater in men) estimates (Sullivan et al. 2003). There has been no twin study that estimated the heritability for either fibromyalgia or chronic widespread pain in adults.

2.5.3 Twin studies of the comorbidity among functional somatic and psychiatric syndromes

Only very few twin studies examined whether somatic symptoms share genetic factors with psychiatric symptoms by using multivariate analysis. Hickie et al. (1999b) examined psychological distress, fatigue, and immune responsiveness in 124 adult twin pairs. Their trivariate analysis indicated that covariation between psychological distress and fatigue was solely explained by genetic factors in common to the measures, and that independent genetic and nonshared environmental factors also influenced fatigue. Recently, Fowler et al. (2006) fitted univariate and bivariate models with depression and short (> 1 week) or prolonged (> 1 month) fatigue to data from 1052 young twin pairs aged 11-17. In their univariate analysis, the authors found a heritable component (67%) for short fatigue, whereas no genetic and significant shared environmental effects were found for prolonged fatigue. The bivariate analysis also suggested the importance of shared environment for the covariation, although the majority of variation was unique to each symptom. To date, no study has examined genetic and environmental influences on the comorbidity of chronic widespread pain and chronic fatigue, or between these and psychiatric disorders.

Another sophisticated way of using twins to examine familial influences is the co-twin control study, in which an affected twin and his or her unaffected co-twin (*discordant* pairs) are compared. The co-twin control method can be considered as a case-control analysis with adjustment for not only observable traits or exposures but also non-measurable confounding such as genetic and family environmental factors. This design is a helpful tool to determine whether the source of covariation between two illnesses or traits is due to familial factors in common. Aaron et al. (2001) found in 127 twin pairs discordant for chronic fatigue a more than 20-fold higher risk for having a lifetime diagnosis of fibromyalgia and a nearly 10-fold higher risk for having irritable bowel syndrome. Roy-Byrne et al. (2002) then found higher scores of psychological distress in 100 fatigued female twins than in their non-fatigued, same-sex co-twins. The results were similar across zygosity, suggesting that the association is more environmental than genetic. In a population-based twin study, Svedberg et al. (2002) performed a two-step analysis: (1) comparing 72 subjects with irritable bowel syndrome and 216 unrelated subjects without the syndrome, and (2) comparing 58 affected twins with their unaffected co-twins. The comparison between the first and second analyses allowed the authors to assess the influences of confounding by unmeasured familial factors on the associations among comorbid conditions. If a significant association is found in the second (i.e., co-twin control) analysis, it would suggest other environmental or causal influences than familial ones on the association. However, the authors found no significant evidence for familial confounding in the comorbidity of irritable bowel syndrome with other functional somatic syndromes or psychiatric disorders.

2.6 WHAT DO WE NEED TO KNOW?

The etiology of functional somatic syndromes and their comorbidity remain elusive. Among others, the following are probably the four most important questions in this area of research. These questions are addressed and tackled throughout the thesis.

2.6.1 What are the sources of familial aggregation?

Once familial aggregation is suggested for an illness, the next step is to examine its sources. As noted in Section 2.4.2, genetically informative samples such as twins are helpful for disentangling the sources of familial aggregation into genetic and family environmental components. To date, no study has estimated the heritability for either fibromyalgia or chronic widespread pain in an adult sample. This is the main aim of Study I. Given the striking female preponderance, sex differences in the estimates should also be examined. Including opposite-sex twin pairs permits testing statistically whether there are sex differences in the type (i.e., sets of genes expressed) or magnitude (i.e., effect sizes of gene expression) of genetic influences.

2.6.2 Why do the syndromes overlap?

In order to examine the justifiability of the concept of functional somatic syndromes, it is necessary to know about possible mechanisms underlying the overlap of the syndromes. As described above, the co-twin control analysis is a powerful tool to assess the source of covariation between comorbid illnesses. A variety of symptoms and conditions purportedly associated with chronic widespread pain are analyzed in Study II. Associations are compared between unrelated, matched case-control pairs and discordant twin pairs to evaluate (1) the extent to which there is comorbidity and (2) the extent to which the comorbidity can be attributed to familial influences or confounding. Because the comorbidity is the core of question in this thesis, it is further investigated in Study IV.

2.6.3 What causes the psychiatric comorbidity?

As pointed out in Section 2.2.3, it has been unclear whether comorbid depression and anxiety precede the occurrence of functional somatic syndromes or whether they are consequences of those painful or fatiguing symptoms. In addition, the risk of stressful events has been inconclusive due to potential biases in previous studies. A prospective study, in which data are obtained from the same individuals at different time points, is required to investigate the direction of a possible causal relationship. By combining the co-twin control design and the longitudinal design, purported associations of personality (extraversion, emotional instability) and perceived stress with chronic fatigue are assessed in Study III. Information about the nature of these associations should help to understand the reason for the comorbidity with psychiatric disorders.

2.6.4 Functional somatic syndromes: one or many?

This as yet unanswered question was raised by Wessely et al. (1999). Despite the intensive disputes reviewed in Section 2.4, no study has provided empirical evidence that the overlap in functional somatic syndromes is attributable to an etiological factor in common. It is also unclear whether the common factor, if any, is psychological or physiological. This is the last and most important question of this thesis, and is scrutinized using a multivariate twin design in Study IV. Four major functional somatic syndromes (chronic widespread pain, chronic fatigue, irritable bowel syndrome, and recurrent headache) and two psychiatric disorders (major depression and generalized anxiety disorder) are analyzed concurrently, by fitting data to a series of models typically used in multivariate twin studies.

3 AIMS

The overall aim of this thesis is to uncover the etiology of functional somatic syndromes and their comorbidity, with special focuses on chronic widespread pain and chronic fatigue.

The specific aims are:

1. To evaluate the relative importance of genetic and environmental influences in liability to chronic widespread pain, including sex differences in the influences.
2. To assess the associations between chronic widespread pain and its comorbid illnesses in the general population, and evaluate the genetic and environmental influences on the comorbidity.
3. To examine the relationship between psychosocial factors and a functional somatic syndrome, chronic fatigue.
4. To determine whether functional somatic syndromes can be attributed to etiological factors in common or should be considered as distinctive entities.

4 METHODS

4.1 PARTICIPANTS

4.1.1 The Swedish Twin Registry

All of the present studies were based on the Swedish Twin Registry (STR; Lichtenstein et al. 2002; Lichtenstein et al. 2006). The STR is one of the oldest and largest twin registries in the world, established in the late 1950's and currently consists of more than 160,000 twins born in Sweden since 1886, with data on date of birth, place of birth, addresses, and current vital status. The registry is updated on a regular basis by matching Swedish national registers such as the Cause of Death Register.

4.1.2 The SALT study

The STR carried out a large-scale, comprehensive screening interview with middle-aged and older twins between 1998 and 2002, known as the Screening Across the Lifespan Twins (SALT) study (Lichtenstein et al. 2002). A pilot study was conducted from 1995 to 1996, in which 1,321 individuals participated through telephone interviews. The full-scale screening was then commenced in March 1998 and was completed in December 2002. All living, contactable, and consenting twins in the STR were invited randomly, approximately 1,000 individuals per month. Efforts were made to contact a twin and his or her co-twin within an interval of 1 month in order to avoid the effect of differential age at testing. The telephone interviews were performed via a computer-based data collection system. Interviewers were trained personnel with an adequate medical background, such as nurses and medical students. All participants provided verbal informed consent during the interview, and consent was later confirmed by postcard. The data collection procedures (including the use of previous studies in the STR) were reviewed and approved by the Swedish Data Inspection Board, Stockholm, Sweden, and the Regional Ethics Committee of the Karolinska Institutet, Stockholm.

4.1.3 Questionnaire 1972-1973

In Study III, information about premorbid risk factors for chronic fatigue was taken from mailed questionnaires administered approximately a quarter-century before the commencement of the SALT study. In 1972-1973, questionnaires were sent out to monozygotic and same-sex dizygotic twins born between 1926 and 1958. It was standard not to study opposite-sex twins in early 1970's because their utility had not yet been widely appreciated. The questionnaire included questions regarding demographic characteristics, health behaviors, environmental exposures, and psychosocial indices.

4.1.4 Zygosity determination

Zygosity of the participants was based on responses to questions regarding physical similarity between members of a pair ("During childhood, were you and your twin partner as like as two peas in a pod?"). This method was validated as having 99% or

higher accuracy in the SALT-pilot study using 13 DNA markers (Lichtenstein et al. 2002).

4.2 ASSESSMENT PROCEDURES

4.2.1 Chronic widespread pain (Study I, II, IV)

The screening algorithm for chronic widespread pain was based on the classification criteria for fibromyalgia proposed by the American College of Rheumatology (ACR; Wolfe et al. 1990). The stem question was, "Have you suffered from general pain during the last three months?" Interviewees who endorsed this item were then asked, "Did you have continuous pain during all three months?" Subjects who endorsed the second item were further asked, "Do you suffer from pain in both the upper and lower body?" and "Do you suffer from pain in both the right and left sides?" Information about axial skeletal pain was also obtained by asking, "Have you had any back pain in the last 12 months?" Subjects who endorsed all four of these items were defined as having chronic widespread pain. Those who did not endorse the first or second question regarding general pain during the last three months were considered to be unaffected and were used as controls in the analyses. In addition, queries about sleeping disturbance, tenderness or stiffness, and working impairment were also made. The ACR's definition requires no exclusionary criteria.

The aforementioned criteria differed from the ACR definition of fibromyalgia in two ways. First, the definition of chronic widespread pain in this study relied on self-reports (without clinical examinations by physicians or inspection of medical records). Second, a count of tender points was not required.

4.2.2 Chronic fatigue (Study II, III, IV)

Chronic fatigue was assessed only for subjects younger than 65 years. The screening module for chronic fatigue in the telephone interview was based on the United States Centers for Disease Control and Prevention consensus criteria for chronic fatigue syndrome (Fukuda et al. 1994). The stem question, "Have you felt abnormally tired during the last six months?" was used to code fatigue. The time frame was the six months prior to interview, as assessment of lifetime fatigue was believed to be considerably less reliable. Subjects who endorsed this item were then asked about the continuousness of fatigue in the prior six months and about the duration of continuous fatigue. Impairment was considered present if subjects believed that fatigue made them too tired to live a normal life, had caused social problems, or had caused work incapacity of 25% or greater. Finally, subjects were asked about eight ancillary symptoms during the period of abnormal tiredness (substantial impairment in short-term memory or concentration; sore throat; tender lymph nodes; muscle pain; multijoint pain without swelling or redness; headaches of a new type, pattern, or severity; unrefreshing sleep; and postexertional malaise lasting more than 24 hours). The presence of four or more of these ancillary symptoms is a component of the definition of chronic fatigue syndrome.

Fatigue was defined as the presence of self-reported abnormal tiredness in the absence of an exclusionary condition. Information about exclusions was obtained from the following sources: (1) the telephone interview (e.g., morbid obesity, lifetime history of an eating disorder); (2) Swedish national registers (e.g., malignant

neoplasm, hospitalization owing to narrow definitions of schizophrenia, schizoaffective disorder, and bipolar disorder); and (3) physician review of all available medical records that revealed the presence of any other exclusionary diagnosis (e.g., drug or alcohol dependence, sleep disorder with adequate workup, infection with hepatitis B or C or human immunodeficiency virus). In the absence of exclusionary conditions, *chronic impairing fatigue* was defined as the presence of fatigue with a duration of six months or longer plus impairment, and *CFS-like illness* was defined as chronic impairing fatigue plus four or more ancillary symptoms.

4.2.3 Irritable bowel syndrome and headache (Study II, IV)

Irritable bowel syndrome was screened by an algorithm used in a prior STR study (Svedberg et al. 2002) which is in line with the Rome criteria (Thompson et al. 1989). In brief, subjects who endorsed experiencing recurrent discomfort in the stomach or intestine at least seven days per month with one of six specific symptoms (e.g., diarrhea or loose feces more than three times per week) were defined as having irritable bowel syndrome.

Recurrent headache, migraine, and tension-type headache were assessed only for subjects younger than 65 years by algorithms used in prior STR studies (e.g., Svensson et al. 2004) and based on the International Headache Society criteria (International Headache Society 1988). Recurrent headache was considered present if the subject endorsed an introductory question, “Do you or have you ever suffered from recurrent headaches, that were not caused by infection, fever, or hangovers?”, followed by questions for migraine and tension-type headache.

4.2.4 Psychiatric disorders or symptoms (Study II, IV)

Major depression and generalized anxiety disorder were assessed by using the Composite International Diagnostic Interview-Short Form adapted from its original design for 12-month prevalence to assess lifetime prevalence (Kessler et al. 1998). These algorithms were used in previous SALT studies (Kendler et al. 2006a; Mackintosh et al. 2006).

Depressive symptoms were assessed using the Iowa Short Form of the Center for Epidemiology Studies Depression Scale (Kohout et al. 1993; Radloff 1977) with 11 items, and a score of nine or above was considered as the presence of depressive symptoms. Eating disorders were assessed for subjects under 65 years of age via questions based on the Structured Clinical Interview for *DSM-IV* (First et al. 1997), which was also used in a prior SALT study (Bulik et al. 2006).

4.2.5 Other comorbid illnesses or conditions (Study II)

Information about possible rheumatoid arthritis was obtained from a single question regarding the presence or absence of current or past rheumatoid arthritis. Possible rheumatoid arthritis was considered present if the subject was diagnosed by a physician as having it in a knee or a hip. Prolonged joint pain was considered present if its duration was more than four weeks at a time. Osteoarthritis was considered present if the subject was diagnosed by a physician as having it in a knee or a hip.

Subjects who endorsed any of these items about pain (i.e., possible rheumatoid arthritis, prolonged joint pain, and osteoarthritis) were considered to have *joint pain*.

Gastroesophageal reflux disease was screened by an algorithm used in a prior STR study (Cameron et al. 2002). Allergy was considered present if the subject affirmed any type of allergy given in a list (e.g., eczema). Subjects were asked to rate their general health and categorized as having good to excellent health versus indifferent to bad health. The body mass index was calculated as the weight in kilograms divided by the square of height in meters. The cutoff point for overweight was 25 or higher. Frequent infections were assessed if the subject usually had common colds or other infections more than twice per year.

4.2.6 Psychological risk factors (Study III)

Stress and personality data were collected as part of the questionnaire administered in 1972 and 1973. Stress was assessed by the question, "Do you experience your daily existence as being very 'stress-filled'?" with the answers coded as yes or no.

Personality was assessed as indexed by measures of extraversion and emotional instability (also known as neuroticism) using a short form of the Eysenck Personality Inventory (Eysenck & Eysenck 1968). Each scale score was based on the sum of yes and no responses to nine items. We used mean imputation if one item for a particular scale was missing. Individuals having more than one missing item for a particular scale were excluded. Raw scale scores were standardized using a regression technique (McGue & Bouchard 1984) to adjust for the effects of age, sex, and age X sex interaction.

4.3 STATISTICAL ANALYSES

The present studies analyzed twins by two different approaches: one was a quantitative genetic design, and the other was a co-twin case-control study design. The former design was used in Study I and IV for univariate and multivariate analyses, respectively, and the latter design was used in Study II and III for cross-sectional and longitudinal analyses, respectively.

4.3.1 Univariate twin analysis (Study I)

4.3.1.1 Concordance rate

The concordance rate is a simple and easy-to-understand way of estimating the extent to which genetic effects are important for a dichotomous variable. There are two forms of the concordance rate, namely pairwise and probandwise. The probandwise concordance rate is a conditional probability that a twin is affected given that his or her co-twin is affected. However, the concordance rate can be biased if ascertainment probabilities are different in co-twins of the affected and those of the unaffected, or in disease-concordant twin pairs and disease-discordant twin pairs (Strachan 2000).

4.3.1.2 Liability-threshold model

Although the observed status of a disease is usually measured simply as a dichotomous variable, i.e., affected versus unaffected, the liability to the disease of interest is often assumed to be quantitative and normally distributed (Falconer & Mackay 1996). This assumption is biologically reasonable because of the inherent polygenic background of complex diseases. The liability is considered to have one or more thresholds in such a way that the area under the normal curve above a particular threshold reflects the prevalence of the disease. This theorization can easily be extended to twin data (Posthuma et al. 2003).

4.3.1.3 Correlations within twin pairs

The similarity within twin pairs can be measured by the intraclass correlation. In the case of a dichotomous variable, the tetrachoric correlation is applied assuming the liability-threshold model. When the correlation is calculated, a "double-entry" of data is performed so that possible biases due to birth order or non-random assignment of twins as 'twin A' and 'twin B' can be avoided.

As a preliminary analysis, the tetrachoric correlations for chronic widespread pain in men and women were computed by PROC CORR in SAS[®] statistical package version 9 (SAS Institute 2005). The values in monozygotic and dizygotic twins were compared in order to obtain a first estimate of the relative contribution of genetic effects to the phenotypic variation of a variable.

4.3.1.4 Structural equation modeling for twin data

Structural equation modeling is an extensively used statistical technique in various field of science including quantitative genetics. The purpose of using this technique for twin data is to decompose the phenotypic variation of a variable into genetic and environmental variances, by comparing the similarities within monozygotic and dizygotic twin pairs. To do this, a few fundamental assumptions are required: (1) Monozygotic twin pairs share 100% of their genes, whereas dizygotic twin pairs share 50% of their segregating genes on average; (2) Monozygotic and dizygotic pairs share their family environment to the same degree (so-called 'the equal environment assumption'). In addition, variances of the variable in question need to be equal across zygosity for precise estimation.

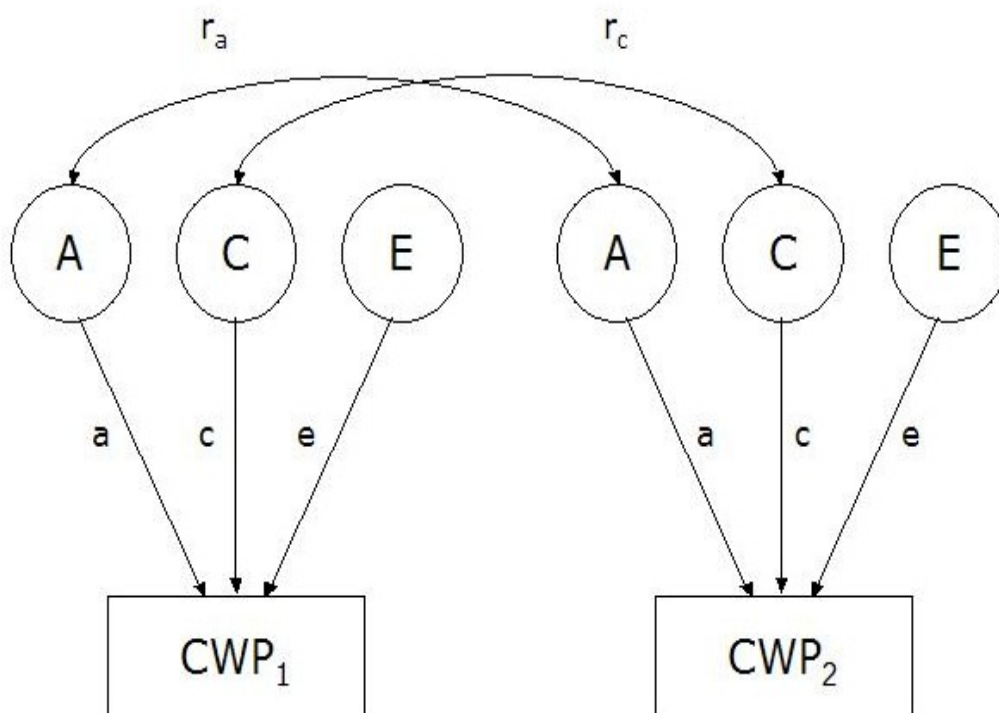
In general, the phenotypic variance is assumed to be due to three latent factors: additive genetic factor (A), shared environmental factor (C), and nonshared environmental factor (E). The last factor also includes measurement error. The correlation for monozygotic twin pairs is assumed to be due to additive genetic and shared environmental factors (A + C), whereas that in dizygotic twins is due to half the genetic plus shared environment ($\frac{1}{2}A + C$). Heritability is defined as the proportion of total phenotypic variance attributable to genetic variance.

Actually, genetic factors do not necessarily act in additive ways. Two nonadditive genetic effects are dominance (i.e., interaction between alleles at a single locus) and epistasis (interaction among genes at different loci). However, dominant genetic and shared environmental effects are negatively confounded and therefore unable to be estimated simultaneously unless more information is included (e.g., reared-apart

twins). The intraclass correlations can be used to determine whether dominant genetic or shared environmental effects should be included in the model. Because the probability of having the same combination of alleles at a particular locus is 25% in dizygotic twin pairs compared to 100% in monozygotic twin pairs, a correlation within dizygotic twin pairs smaller than half of that within monozygotic twin pairs suggests the importance of dominance effects.

Figure 1 illustrates a typical path diagram used in univariate twin analysis, which is called ‘ACE model’. This diagram is mathematically equivalent to a set of structural equations. Parameters were estimated using a maximum likelihood procedure for raw data implemented in the Mx program (Neale et al. 2004), a computer software specifically tailored for quantitative genetic analyses. The Mx program has some advantageous features such as (1) being available online without cost; (2) being capable of fitting to raw data; and (3) providing easy ways to compare a given model and its submodels using a traditional χ^2 statistic. Twice the difference between the log-likelihood of a full model and that of a nested submodel is asymptotically χ^2 distributed with the difference in the number of estimated parameters as the degrees of freedom. Thus, a significant difference in the goodness-of-fit indicates that the model with fewer estimated parameters fits the data worse.

Figure 1. A path diagram for univariate analysis (ACE model).



A: additive genetic factors; C: shared environmental factors; E: nonshared environmental factors;
 r_a : genetic correlation; r_c : environmental correlation.

4.3.1.5 Sex-limitation model

Data including opposite-sex dizygotic twins enable one to test the two assumptions used for same-sex dizygotic twins (Neale & Martin 1989). If the genetic influences for a trait are different in men and women, opposite-sex twin pairs will be less genetically similar than same-sex dizygotic twin pairs, i.e., $r_a < 0.5$ in Figure 1 (Suppose that CWP_1 is male and CWP_2 is female). Similarly, if the family environmental influences are different in men and women, the environmental correlation within opposite-sex twin pairs will be smaller than that within same-sex dizygotic twins, i.e., $r_c < 1.0$. Thus, significantly worse goodness-of-fits when r_a and r_c are fixed at 0.5 and 1.0, respectively, suggest that different sets of genes or shared environment are important for men and women. Further, parameter estimates were constrained to be equal in men and women, using both same-sex and opposite-sex twins. A significant difference in the goodness-of-fit indicates that the relative importance of variance components differs between men and women.

4.3.2 Multivariate twin analysis (Study IV)

4.3.2.1 Multivariate models for comorbidity

Univariate twin analyses can easily be extended to a multivariate model when more than one measure has been assessed for each individual. Multivariate analysis allows the decomposition of an observed correlation between two variables into a genetic and an environmental component. This can be quantified by calculating the genetic and environmental correlations and the genetic and environmental contributions to the observed correlation.

There are three commonly used multivariate factor models in twin studies: the Cholesky decomposition (or triangle decomposition), the independent pathways (or biometric) model, and the common pathways (or psychometric) model (Neale & Maes 2004). These models are described in the following three subsections. The goodness-of-fits of these models can be compared by using their Akaike Information Criterion (AIC; Akaike 1987), which represents the balance between the fit and the parsimony of the model. A lower value of AIC indicates a better model than one with a higher AIC.

4.3.2.2 Cholesky decomposition

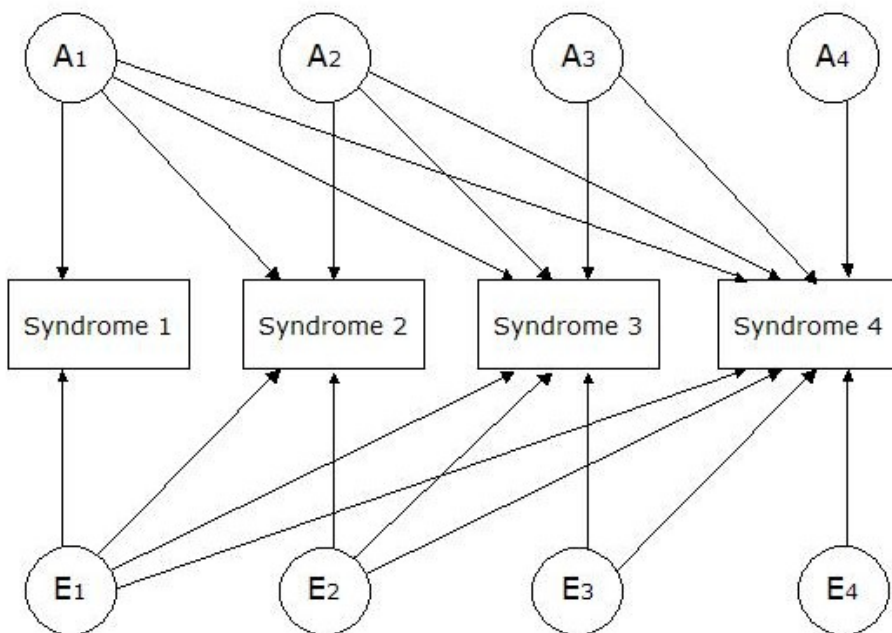
Cholesky decomposition (Figure 2a) is the most fully parameterized model. The first factor A_1 loads on all the variables, the second factor A_2 loads on all but the first one, the third factor A_3 loads on all but the first two, and so on. The Cholesky model is typically used as a saturated model against which the fits of more constrained models are compared.

4.3.2.3 Independent pathway model

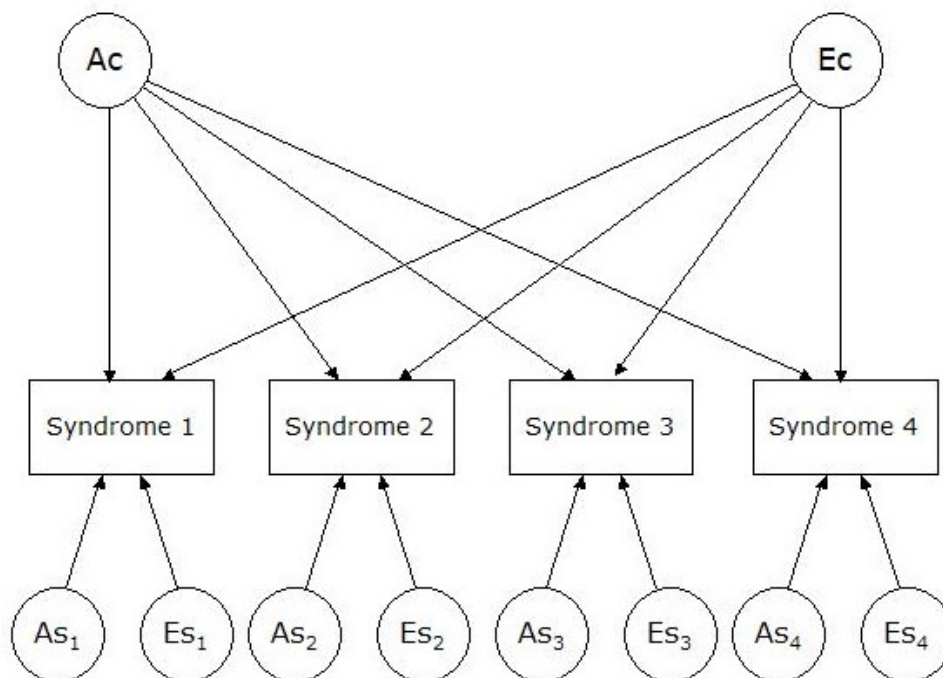
Independent pathway model (Figure 2b) assumes a common genetic factor (A_c) and a common environmental factor (E_c) that affect all the observed variables directly. Each observed variable may have a residual variance, which can also be partitioned into genetic and environmental components specific to each variable (A_s and E_s).

Figure 2. Models for underlying structures of comorbidities (quadrivariate AE models).

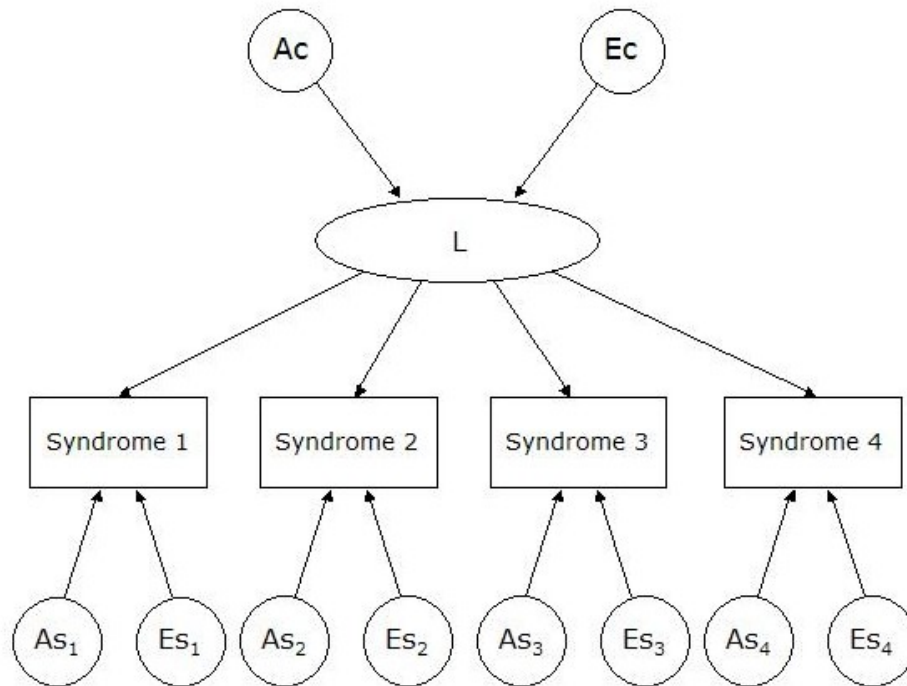
(a) Cholesky model



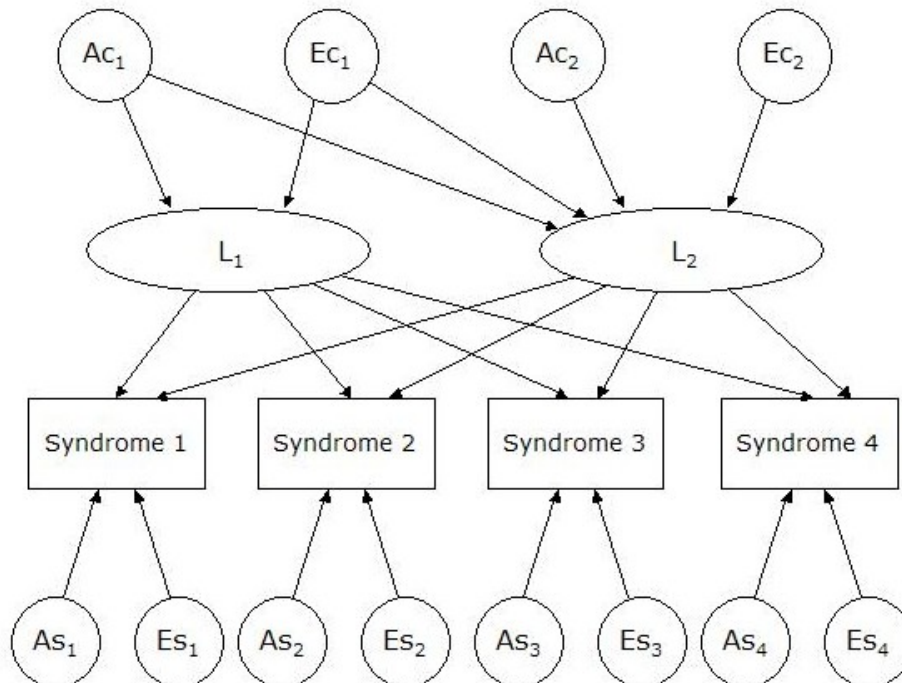
(b) Independent pathway model



(e) Common pathway model (1-factor)



(d) Common pathway model (2-factor)



A: additive genetic factors; E: nonshared environmental factors. L: latent factor. Factors with a subscript "c" are those in common with all syndromes included, whereas factors with a subscript "s" are specific to each syndrome. For simplicity, only one twin of a pair is shown and shared environmental factors are excluded.

4.3.2.4 Common pathway model

Common pathway model with one factor (Figure 2c) assumes a common, latent factor (L) that affects all the observed variables. In this case, genetic and environmental factors (Ac and Ec) exert their influences on the variables through this latent factor. As in the independent pathway model, each variable is allowed to have a residual variance that can be partitioned into specific components (As and Es).

Common pathway model can be extended to the two-factor solution (Figure 2d) when at least four or more observed variables are included. Three-factor solution requires at least six variables to make it identifiable.

4.3.3 Co-twin case-control analysis (Study II, III)

4.3.3.1 Design

The purpose of any type of matched design is to control for confounding. For this purpose, comparison between a twin affected for the illness of interest and his or her unaffected co-twin can be considered a variant of the matched case-control study. Pairs of twins share not only measurable characteristics such as age, sex, and place of birth but also factors that may not be measured, such as genetic make-up and family environment early in life. In particular, monozygotic twinning is an ideal natural experiment where 100% genetic background can be controlled for. Actually, one of the original aims in setting up the large twin registries in Scandinavian countries was to collect illness and exposure discordant twin pairs, allowing an analysis of environmental risks for illness while controlling for genetic factors (e.g., Cederlof et al. 1977). Unlike usual epidemiological research in which genetic factors are often treated as noise or nuisance, to detect familial (genetic and family environmental) confounding has a positive meaning in the co-twin control study. In this, the concept of “exposure” is extended to genes.

4.3.3.2 Generalized estimating equations (GEE)

In order to detect familial confounding, we first need to evaluate the strength of association of interest without adjustment by using unrelated, matched case-control analysis. In the present study, this was carried out by a statistical technique named generalized estimating equations (GEE) using PROC GENMOD in SAS. Non-independence of twin pairs in the data is treated in GEE analysis, resulting in estimates mathematically equivalent to classical matched case-control analysis. An advantage of GEE relative to matched case-control analysis is that GEE utilizes all of the non-case observations in the computation.

4.3.3.3 Conditional logistic regression

The co-twin control analysis, which is a special case of matched case-control analysis, was performed using PROC LOGISTIC in SAS. We employed two steps: first, both monozygotic and same-sex dizygotic twins discordant for the illness of interest were included; then, only discordant monozygotic twins were used. In the latter, not only family environment but also genetic factors can be perfectly controlled

for. A drop in the strength of association between GEE and co-twin control analysis reflects familial confounding. A significant association when using only monozygotic twins indicates the importance of environmental effects unique to each individual (i.e., nonshared environment). If the association is with an exposure preceding disease, inferences about causal associations may be cautiously offered.

4.3.3.4 Prospective study

In Study III, the information about exposure (personality traits and perceived stress) was collected in 1972-73, which was more than 25 years before the information about the health outcome (chronic fatigue) was assessed in 1998-2002. Because a question about the duration of fatigue was included in the telephone interview, we could identify subjects who already had the symptom at the time the questionnaire was administered. These subjects were excluded from the subsequent analysis in Study III.

5 RESULTS

5.1 DESCRIPTIVE STATISTICS

Of 61,355 twins who were eligible for the SALT study, 44,897 (73.2%) responded to the telephone interview. The mean \pm SD age of the respondents was 59.8 ± 11.1 years, and 53.5% were women. Both members of 15,950 twin pairs responded to the interview items regarding pain symptoms; 4,170 of these pairs were monozygotic, 5,881 were same-sex dizygotic, 5,755 were opposite-sex dizygotic, and 144 were of unknown zygosity. The pairs for whom zygosity was unknown were excluded from the analyses. Of 44,897 participants, 31,318 were under 65 years of age.

Table 1 shows the prevalence rates of four functional somatic syndromes (chronic widespread pain, chronic fatigue, irritable bowel syndrome, and recurrent headache) and two psychiatric disorders (generalized anxiety disorder and major depression). Significant sex differences in prevalence estimate were found for all of the six illnesses.

Table 1. Sex-specific estimates of prevalence for the 6 illnesses in the participants aged 41 or over.

Illness		CWP	CF*	IBS	RH*	GAD	MD
Number of respondents	Women	23,153	16,278	22,286	16,362	24,029	23,178
	Men	20,185	14,710	19,055	14,780	20,868	20,198
Number of cases	Women	1,406	1,312	1,722	5,330	1,009	5,703
	Men	370	511	870	2,625	430	2,770
Prevalence [†] (%)	Women	6.1	8.1	7.7	32.6	4.2	24.6
	Men	1.8	3.5	4.6	17.8	2.1	13.7

CWP: chronic widespread pain, CF: chronic impairing fatigue, IBS: irritable bowel syndrome, RH: recurrent headache, GAD: generalized anxiety disorder, MD: major depression.

Subjects who did not provide usable answers for screening were excluded.

*Only subjects under 65 years of age were included.

[†]Differences in prevalence between women and men were all statistically significant ($P < .0001$) by *t* test.

5.2 HERITABILITY ESTIMATES FOR CHRONIC WIDESPREAD PAIN (STUDY I)

Table 2 shows indicators of intrapair similarity according to zygosity and sex. Monozygotic twins were more similar than dizygotic twins, suggesting the importance of genetic influences and negligible influences of shared environment. The pattern of similarity across zygosity did not suggest strong evidence for nonadditive genetic effects.

There was no significant change in goodness-of-fit when the genetic and shared (family) environmental correlations for opposite-sex twins were constrained to 0.5 and 1.0, respectively ($\Delta\chi^2 = 0$, $\Delta df = 2$, $P = 1.0$), suggesting that the same genetic and shared family environmental influences are operating in men and women. Table 3 summarizes the resulting parameter estimates based on the best-fit model. Modest genetic influences were observed for both women and men, although the genetic parameter estimate was not statistically significant for men. When the parameters were equated across sex, the goodness-of-fit was not significantly worse than that in separate estimation ($\Delta\chi^2 = 1.09$, $\Delta df = 2$, $P = 0.58$). Estimates were also compared across different age groups (41-54, 55-64, 65+), resulting in no significant differences (data not shown).

Table 2. Numbers of concordant and discordant twin pairs, probandwise concordance rates, and tetrachoric correlations for chronic widespread pain (Kato et al. 2006a).

	Concordant unaffected pairs	Discordant pairs	Concordant pairs	Probandwise concordance rate	Tetrachoric correlation
MZ					
Women	1,907	177	37	0.29	0.55
Men	1,638	50	4	0.14	0.46
DZ					
Women	2,559	307	29	0.16	0.29
Men	2,380	79	2	0.05	0.20
DZO	4,812	407	17	0.08	0.15

MZ: monozygotic twins; DZ: same-sexed dizygotic twins; DZO: opposite-sexed dizygotic twins.

Table 3. Estimates of proportions of variances (with 95% confidence intervals) and goodness-of-fit parameters for chronic widespread pain in men and women (Kato et al. 2006a).

	Proportions of variances* (95% confidence interval)			Goodness of fit	
	Additive genetic	shared familial environment	nonshared environment	-2LL	df
Women	0.54 (0.23 – 0.66)	0.03 (0 – 0.27)	0.43 (0.34 – 0.54)	10549.7	30692
Men	0.48 (0 – 0.67)	0.01 (0 – 0.49)	0.51 (0.33 – 0.76)		

* Estimates were adjusted for age at interview. -2LL: -2 times log-likelihood; df: degrees of freedom.

5.3 COMORBIDITIES ASSOCIATED WITH CHRONIC WIDESPREAD PAIN (STUDY II)

Table 4 shows the ORs of having each comorbid illness or characteristic in cases relative to controls, by using (i) matched case-control pairs (GEE analysis), (ii) monozygotic and same-sex dizygotic co-twins, and (iii) only monozygotic co-twins. In GEE, we found significant ORs for all of the comorbidities, of which chronic fatigue showed the highest. ORs greater than five were also observed for poor self-rated health, joint pain, depressive symptoms, and irritable bowel syndrome. The prevalence and ORs did not differ significantly when restricted to the pairs in which both members of the pair responded. When co-twin controls were used, ORs were reduced in almost all comorbidities and health conditions except migraine, tension-type headache, and frequent infections. Notable reductions from significant association in GEE to non-significance in co-twin control analyses were found for psychiatric disorders (major depression, generalized anxiety disorder, and eating disorders), and overweight. The changes in OR across the degree of adjustment suggest the presence of confounding from genetic and family environmental effects. Nevertheless, the ORs for most of the comorbidities were still significant in the analyses using only monozygotic twins, indicating that these comorbidities are not solely a function of genetic and family environmental mechanisms.

Table 4. Odds ratios and 95% confidence intervals of the associations between chronic widespread pain and its comorbid illnesses or symptoms (Kato et al. 2006b, modified).

Illness or symptom	(i) GEE	(ii) Co-twin (MZ + DZ)	(iii) Co-twin (MZ)
Chronic fatigue (age ≤ 64)			
Chronic impairing fatigue	10.15 (8.90 – 11.58)	7.20 (4.51 – 11.49)	3.71 (2.06 – 6.70)
CFS-like illness	23.53 (19.67 – 28.16)	14.43 (6.71 – 31.04)	9.75 (3.48 – 27.28)
Joint pain and headache			
Joint pain*	7.41 (6.70 – 8.21)	5.18 (3.75 – 7.16)	4.60 (2.63 – 8.04)
Recurrent headache (age ≤ 64)	3.54 (3.17 – 3.95)	3.04 (2.24 – 4.11)	2.64 (1.61 – 4.31)
Migraine (age ≤ 64)	3.04 (2.68 – 3.45)	3.55 (2.35 – 5.36)	3.27 (1.67 – 6.43)
Tension-type headache (age ≤ 64)	2.14 (1.85 – 2.49)	2.79 (1.83 – 4.27)	3.00 (1.47 – 6.14)
Psychiatric disorders			
Current depressive symptoms	5.26 (4.75 – 5.82)	3.14 (2.33 – 4.23)	2.00 (1.27 – 3.15)
Lifetime major depression	2.09 (1.89 – 2.32)	1.51 (1.16 – 1.96)	1.13 (0.74 – 1.72)
Lifetime generalized anxiety disorder	3.07 (2.59 – 3.64)	2.04 (1.25 – 3.33)	1.60 (0.73 – 3.53)
Lifetime eating disorder (age ≤ 64)	1.62 (1.44 – 1.81)	1.08 (0.81 – 1.44)	0.93 (0.59 – 1.45)
Gastrointestinal disorders			
Irritable bowel syndrome	5.17 (4.55 – 5.88)	3.78 (2.47 – 5.77)	3.50 (1.84 – 6.65)
GERD	3.78 (3.42 – 4.17)	2.85 (2.13 – 3.82)	2.17 (1.33 – 3.56)
General health			
Allergy, any	2.17 (1.97 – 2.40)	1.82 (1.40 – 2.38)	1.70 (1.08 – 2.67)
Overweight (BMI ≥ 25)	1.60 (1.45 – 1.77)	1.30 (0.98 – 1.72)	1.28 (0.76 – 2.16)
Poor general health	15.34 (13.48 – 17.46)	7.08 (5.08 – 9.85)	6.20 (3.59 – 10.70)
Frequent Infections (> 2 / yr)	3.34 (2.86 – 3.90)	3.17 (2.00 – 5.01)	3.08 (1.61 – 5.91)

*Joint pain was defined as having at least one of possible rheumatoid arthritis, prolonged joint pain, or osteoarthritis. GEE: generalized estimating equations; MZ: monozygotic twins; DZ: dizygotic twins; GERD: gastroesophageal reflux disease; BMI: body mass index.

5.4 PREMORBID RISK FACTORS FOR CHRONIC FATIGUE (STUDY III)

Of 19,150 monozygotic and same-sex dizygotic participants in the SALT interview aged 41-64, 17,168 (90.0%) had responded to the questionnaire in 1972-73. Table 5 shows standardized scores for personality scales and the proportion of subjects who endorsed their daily life as stressful, comparing the affected and unaffected with the two definitions of chronic fatigue under 65 years of age. Subjects with chronic fatigue had significantly higher scores of emotional instability and significantly lower scores of extraversion relative to subjects without fatigue (*t* test, $P < .0001$). Similarly, fatigued subjects were more likely to experience their life as stressful than controls subjects (χ^2 test, $P < .0001$).

Table 5. Mean standardized scores of personality scales and the proportion of subjects with stress in cases and unaffected controls (Kato et al. 2006c, modified)

Case definition	Emotional Instability [†]		Extraversion [†]		Stress [‡]	
	Cases*	Controls	Cases*	Controls	Cases*	Controls
Chronic impairing fatigue	0.43 (1.08)	-0.09 (0.96)	-0.16 (0.98)	0.03 (1.00)	22.8%	13.3%
CFS-like illness	0.55 (1.10)	-0.09 (0.96)	-0.18 (1.05)	0.03 (1.00)	23.1%	13.3%

Data are mean personality scores (with standard deviation) and proportions of subjects who endorsed their daily life as stressful. Raw personality scores (0-9 measured by the short form of Eysenck Personality Inventory) were standardized on like-sexed respondents (N = 19,150) adjusting for age at the questionnaire, sex, and age-sex interaction by using a regression technique. After the standardization, each score has the grand mean of 0 and standard deviation of 1.

*Subjects who reported having fatigue at the time of the questionnaire (42 individuals) were excluded.

[†] Mean difference between cases and controls was statistically significant ($P < .0001$) by *t* test.

[‡] Difference in proportion between cases and controls was statistically significant ($P < .0001$) by χ^2 test.

Table 6 shows the results of matched case-control analyses using GEE, co-twin control analyses using both monozygotic and dizygotic twins, and co-twin control analyses using monozygotic twins only. In GEE analyses, both definitions of chronic fatigue were significantly associated with emotional instability. These results can be thought of as the same as analyses of an unselected population, and they indicate that there is a 55 to 72% increase in the risk of these definitions of chronic fatigue with

each standard deviation increase in emotional instability (after correction for stress and extraversion). In the second series of analyses, using monozygotic and dizygotic twins, the point estimates dropped only slightly and were still significant. In the final set of analyses, based on monozygotic twins only, the point estimates approached 1.0 and neither definition was significant. These results indicate that the association between emotional instability and chronic fatigue primarily reflects genetic factors that are important for both emotional instability and fatigue. The emotional instability X stress interaction term was not significant in any of the analyses.

Extraversion was not associated with either case definition in any of the analyses in Table 6. Self-reported stress displayed an interesting pattern of results. In the matched case-control analyses, stress was a modest predictor of chronic impairing fatigue; those experiencing their life as stressful in 1972-73 had a 64% to 65% greater risk of developing fatigue later in life. For CFS-like illness, however, risk estimates increased with increasing degrees of adjustment for family environmental and genetic factors. Thus, when genetic influences are controlled, the impact of premorbid stress becomes more pronounced. This suggests that some genes may serve as a buffering effect whereas other sensitive individuals are more susceptible to the impact of stress.

Table 6. Odds ratios and 95% confidence intervals of the associations between personality traits, stress, and chronic fatiguing illnesses (Kato et al. 2006c, modified).

	(i) GEE	(ii) Co-twin (MZ + DZ)	(iii) Co-twin (MZ)
(a) Emotional instability			
Chronic impairing fatigue	1.55 (1.44 – 1.67)	1.49 (1.23 – 1.80)	1.08 (0.77 – 1.51)
CFS-like illness	1.72 (1.53 – 1.93)	1.71 (1.27 – 2.31)	1.02 (0.62 – 1.68)
(b) Extraversion			
Chronic impairing fatigue	0.94 (0.88 – 1.01)	0.98 (0.83 – 1.16)	0.89 (0.65 – 1.22)
CFS-like illness	0.98 (0.88 – 1.09)	0.89 (0.64 – 1.23)	0.91 (0.53 – 1.57)
(c) Stress			
Chronic impairing fatigue	1.65 (1.34 – 2.04)	1.56 (1.01 – 2.43)	1.46 (0.73 – 2.93)
CFS-like illness	1.64 (1.17 – 2.30)	2.44 (1.05 – 5.64)	5.81 (1.21 – 27.85)

GEE: generalized estimating equations; MZ: monozygotic twins; DZ: dizygotic twins; CFS: chronic fatigue syndrome. See Table 5 for the descriptions of psychosocial factors.

5.5 GENETIC AND ENVIRONMENTAL STRUCTURE FOR THE COMORBIDITIES (STUDY IV)

Table 7 shows phenotypic (tetrachoric) correlations among the four functional somatic syndromes and two psychiatric disorders in subjects 41-64 years of age, stratified by sex. The pattern of correlations was generally similar in men and women. The strongest associations were between chronic widespread pain and chronic impairing fatigue, and between generalized anxiety disorder and major depression. Most other correlations were moderate, and all were significantly greater than zero ($P < .001$). Due to the scarcity of male pairs concordant for two of the syndromes, only women were used in the subsequent analyses.

Table 7. Tetrachoric correlations among six illnesses by zygosity in women (N = 16,440) and men (14,878) aged 41-64. (Kato et al. submitted).

	Men	CWP	CF	IBS	RH	GAD	MD
Women							
CWP			0.51	0.34	0.31	0.32	0.23
CF		0.59		0.33	0.29	0.39	0.38
IBS		0.42	0.37		0.24	0.31	0.24
RH		0.36	0.29	0.23		0.23	0.22
GAD		0.25	0.30	0.24	0.16		0.53
MD		0.20	0.27	0.22	0.22	0.49	

CWP: chronic widespread pain, CF: chronic impairing fatigue, IBS: irritable bowel syndrome, RH: recurrent headache, GAD: generalized anxiety disorder, MD: major depression. All correlations are statistically significant at $P < .001$.

Table 8 summarizes the results of model fitting in the multivariate twin analysis of the 6 illnesses. In a series of analyses, models were first tested by dropping shared family environmental (C) factors, resulting in no significant changes in fit. Thus, only additive genetic factors (A) and nonshared environmental factors (E) were included in the models thereafter ('AE models'). When the five models were compared in terms of AIC, the 2-factor common pathway model fit best, i.e., had the smallest AIC. Non-significant paths were further dropped from the 2-factor common pathway model in order to obtain the best-fit model with the most parsimony. Dropping the paths from Ac_1 and Ec_1 to the second latent factor L_2 did not significantly worsen the goodness-of-fit, indicating that these 2 latent factors are independent of each other both genetically and environmentally.

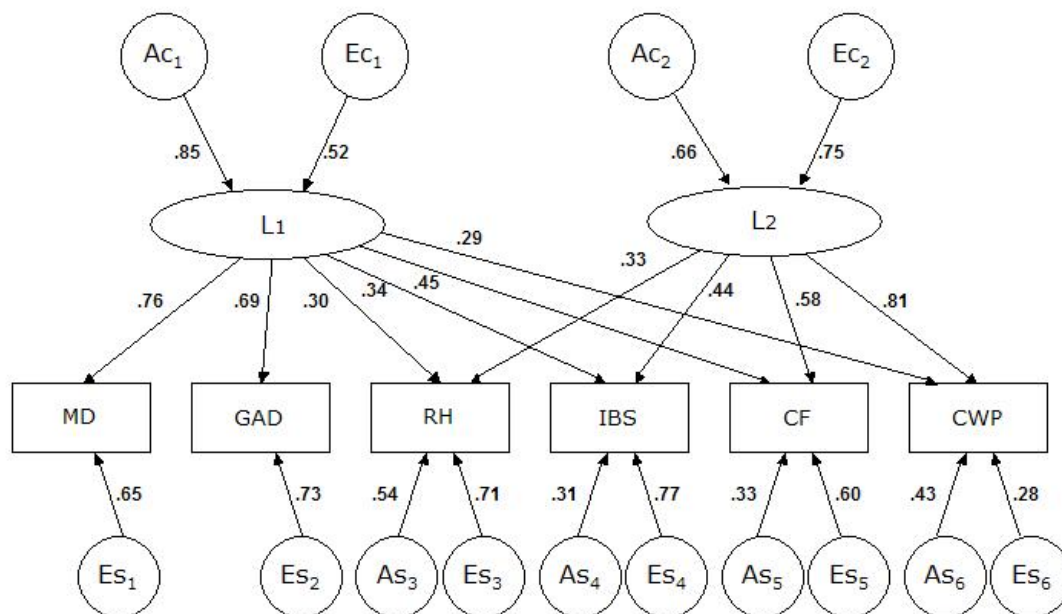
Table 8. Comparison of the goodness-of-fits of 4 types of AE models (Kato et al. submitted).

Type of model	-2 log-likelihood	Degrees of freedom	AIC
Cholesky model	36186.052	49726	-63265.948
Independent pathway model	36212.874	49738	-63263.126
1-factor common pathway model	36392.842	49743	-63093.158
2-factor common pathway model	36149.033	49734	-63318.967
3-factor common pathway model	36140.913	49723	-63305.087

AIC: Akaike Information Criterion. Only women's data were analyzed.

Figure 3 illustrates the best-fit model (2-factor common pathway model) to explain the genetic and environmental structure of the comorbidity. The first latent factor L_1 was more greatly influenced by genetic factor Ac_1 than by environmental factor Ec_1 , whereas L_2 was slightly more influenced by environmental factor Ec_2 than by genetic factor Ac_2 . Only L_1 loaded on major depression and generalized anxiety disorder. In addition, paths from unique genetic factors to these disorders were not significant, suggesting that all the genetic influences on major depression and generalized anxiety disorder can be accounted for by the common genetic factor Ac_1 through L_1 . On the other hand, L_2 loaded primarily on chronic widespread pain, followed by chronic impairing fatigue. For all the four functional somatic syndromes, the loading from L_2 was greater than that from L_1 .

Figure 3. Best-fit (2-factor common pathway) model with age-adjusted parameter estimates for the 6 illnesses in women aged 41-64 (Kato et al. submitted).



A: additive genetic factors; E: nonshared environmental factors. Factors with a subscript “c” are those in common with all illnesses included, whereas factors with a subscript “s” are specific to each illness. L_1 , L_2 : latent factors. MD: major depression. GAD: generalized anxiety disorder. RH: recurrent headache. IBS: irritable bowel syndrome. CF: chronic impairing fatigue. CWP: chronic widespread pain. The total variances of L_1 and L_2 as well as each variable were fixed at 1. Non-significant paths were dropped from the model. For simplicity, only one twin of a pair is shown. Estimates were adjusted for age at the time of interview.

Table 9 summarizes the proportions of total variance of the six illnesses attributable to genetic and environmental factors included in the best-fit model (Figure 3). For example, the proportion of total variance of chronic widespread pain explained by the two latent traits $L_1 + L_2$ can be calculated by adding the square of the path coefficient from L_1 to CWP (0.29^2) plus that from L_2 to CWP (0.81^2), which is 74%. Similarly, the proportion of total variance of chronic widespread pain explained by the genetic effects on L_2 can be calculated by adding the square of the path coefficient from Ac_2 to L_2 (0.66^2) multiplied by that from L_2 to CWP (0.81^2), which is 29%. Note that the total variance of each variable was standardized to be unity, e.g., $0.29^2 + 0.81^2 + 0.43^2 + 0.28^2 = 1.0$ for CWP.

Table 9. Proportions (%) of total variance explained by factors estimated in the best-fit model.

Illness		CWP	CF	IBS	RH	GAD	MD
Latent factor L_1	Ac_1	6	15	8	7	34	42
	Ec_1	2	5	3	2	13	16
	$Ac_1 + Ec_1$	8	20	11	9	47	58
Latent factor L_2	Ac_2	29	14	9	5	0	0
	Ec_2	37	19	11	6	0	0
	$Ac_2 + Ec_2$	66	33	20	11	0	0
Total (Genetic)	$L_1 + L_2$	74	53	31	20	47	58
	$Ac_1 + Ac_2$	35	29	17	12	34	42
Specific factors	As	18	11	10	29	0	0
	Es	8	36	59	51	53	42
Heritability	$Ac_1 + Ac_2 + As$	53	40	26	40	34	42

CWP: chronic widespread pain, CF: chronic impairing fatigue, IBS: irritable bowel syndrome, RH: recurrent headache, GAD: generalized anxiety disorder, MD: major depression. A: additive genetic factors; E: nonshared environmental factors. Factors with a subscript “c” are those in common with all illnesses included, whereas factors with a subscript “s” are specific to each illness. Only women’s data were analyzed. Estimates were adjusted for age at the time of interview.

The extent to which the latent traits L_1 and L_2 account for the total variance varied from illness to illness, ranging from 20% (headache) to 74% (chronic widespread pain). The heritability for chronic widespread pain in women estimated here was consistent with that in the univariate analysis in Table 3, which was 54%. The heritability for chronic fatigue in women estimated here was also comparable with that in a previous study in

SALT (Sullivan et al. 2005a), which was reported as 37% ('CF-B' in their terminology). Genetic effects on the two latent traits ($Ac_1 + Ac_2$) accounted for approximately 70% of the total genetic variance ($Ac_1 + Ac_2 + As$) in chronic widespread pain, chronic fatigue, and irritable bowel syndrome, whereas only 30% in headache. The latent trait L_2 has an eight-fold greater impact (66%) on chronic widespread pain than L_1 (8%), but less than twice on the other three syndromes.

6 DISCUSSION

6.1 INTERPRETATIONS AND IMPLICATIONS OF THE FINDINGS

6.1.1 The two-component model of functional somatic syndromes

The highlight of this thesis is the finding that the origins of comorbidity among functional somatic syndromes are likely composed of two latent traits in common to the syndromes (Figure 3). One of the traits (L_1) predominantly reflects psychiatric disorders and is primarily influenced by genetic factors. In contrast, the other trait (L_2) does not load on psychiatric disorders but rather on all the syndromes, particularly chronic widespread pain, and is primarily influenced by environmental factors unique to the individual. Furthermore, each syndrome has its own genetic and environmental influences specific to each. The extent to which the illness-specific variation is explained by the common traits and by specific factors varies from illness to illness. The majority of influences specific to each illness were nonshared environment with the exception of chronic widespread pain, for which most of the variation was genetic effects. Finally, the two latent traits do not share underlying genetic or environmental influences. Indeed, this etiological model answers almost all the research questions posed in the aims of this thesis. Without regard to the order in which the questions were presented in the BACKGROUND (Section 2.6), this section first proposes the “two-component model” described here as a possible solution to the controversy over the comorbidity of functional somatic syndromes, and then discusses how the findings can be interpreted by the model.

Classically, pain has been considered a multi-dimensional experience including sensory and affective aspects (Price et al. 1987), and as such, the International Association for the Study of Pain defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey et al. 1986). Studies on underlying structure in pain descriptors and pain responses using multivariate statistical approaches have almost always found sensory and affective aspects (Fernandez & Turk 1992). In addition, evidence in experimental studies suggests that sensory and affective aspects of pain are separable and are modulated by different regions in the brain (Rainville et al. 1997; Rainville et al. 1999). Given the distinct, affective and non-affective nature of the latent traits L_1 and L_2 and their patterns of loadings on the comorbid illnesses (many of which share pain as a common symptom), it is reasonable to interpret that L_1 and L_2 reflect affective and sensory aspects of functional syndromes, respectively.

In fact, not only pain sensation and perception but pain amplification and persistence may also have affective and physiological mechanisms (Dubner & Ren 1999; Zusman 2002). It is known that intensive and persistent stimulation of neurons can lead to changes in function and eventually in structure of the central nervous system, i.e., neuronal plasticity (Woolf & Salter 2000), and that such changes can result in central sensitization. As mentioned earlier, central sensitization has been proposed as a potential common denominator of functional somatic syndromes and their comorbid illnesses (Yunus 2005). In patients with fibromyalgia, repetitive nociceptive input originating in peripheral tissues may result in central sensitization (Price & Staud 2005). Pain is modulated by the central nervous system via descending pathways, involving both inhibition and facilitation (Ren & Dubner 2002). An imbalance of the inhibitory and facilitatory modulation may lead to persistent pain. In addition, pain facilitation is known to include affective factors (Price 2000) and the limbic system

may play a pivotal role (Casey 1999; Hutchison et al. 1999). Collectively, it is tempting to speculate that the L_1 and L_2 capture affective and physiological pathways, respectively, of pain regulatory or modulatory mechanisms in the central nervous system.

In light of the view introduced here, the two-component model seems to encompass some of the previously postulated hypotheses such as affective spectrum disorder (Hudson & Pope 1990) and central sensitivity syndromes (Yunus 2000). In other words, these hypotheses may be considered as sub-models of the two-component model. A noteworthy advantage of the present model compared to previous hypotheses is that this model has been derived empirically, using a large population-based sample assessed by blinded, structured interviews. The final best-fit model was selected through a series of statistical tests in a conventional manner, which is free from a priori knowledge or biases due to investigators' medical specialty (Wessely et al. 1999; Wessely & White 2004). In the next two sections, both of the traits are discussed, followed by further deliberation of what the model argues over the current views on functional somatic syndromes.

6.1.2 Possible mechanisms for the psychiatric comorbidity

The first latent trait L_1 in Figure 3 loaded on all of the six illnesses examined, i.e., four functional somatic syndromes and two psychiatric disorders. Because the psychiatric disorders were not influenced by the second latent component L_2 , the comorbidity with psychiatric disorders was mediated solely by L_1 . Even more interestingly, neither major depression nor generalized anxiety disorder was influenced by specific genetic factors A_s . That means the latent genetic factor Ac_1 reflects all the genetic influences on the etiology of these psychiatric disorders. Previous twin studies have found that liability to lifetime major depression and generalized anxiety disorder share genetic factors in their pathogenesis (Kendler et al. 1992; Roy et al. 1995). In other words, it is environmental influences that distinguish between these two disorders. This is also consistent with the results in the co-twin case-control analyses (Study II) shown in Table 4, where the two- to three-fold associations between chronic widespread pain and major depression or generalized anxiety disorder in the matched case-control analysis (GEE) lost their statistical significance when using only monozygotic twins.

Chronic fatigue was more related to L_1 compared to the other three functional syndromes (Table 9). Although it is incorrect to say that the *absolute* impact of psychiatric characteristics on chronic fatigue is greater than that on others, the *relative* importance can be regarded as greater for chronic fatigue. It is therefore rational to focus on chronic fatigue in order to evaluate the relationship between psychological factors (personality and stress) and functional somatic syndromes. As expected, the prospective matched case-control analysis (Study III) shown in Table 6 demonstrated that perceived stress and emotional instability assessed a quarter century ago predict the likelihood of having chronic fatigue. Subjects with pre-morbid self-reported stress or emotional instability had 65% greater risk for the occurrence of chronic fatigue per unit change in standard deviation. Extraversion did not predict chronic fatigue. It is of note that these associations were estimated with mutual adjustment, that is, the impact of stress on chronic fatigue was independent of the level of emotional instability and vice versa. In other words, the confounding effect

by emotional instability was excluded from the association between stress and chronic fatigue.

Previous studies based on cross-sectional and retrospective analysis have not been able to resolve whether stress and emotional distress precede functional symptoms or syndromes (Raphael et al. 2004b). The present results provide evidence that the presence of perceived stress or emotional instability in the absence of fatiguing symptoms precedes the onset of the symptoms. Similar prospective associations were also found in other analyses of the SALT data (Charles, Gatz, Kato, & Pedersen, unpublished manuscript), in which high scores of emotional instability could predict not only chronic fatigue but also chronic widespread pain, headache, irritable bowel syndrome, gastroesophageal reflux disorder, and stomach or duodenal ulcer. Because all of these illnesses were endorsed as lasting for significant periods of time, the prospective associations suggest fundamental roles of emotional instability in the etiology of the disorders rather than reflecting excessive focusing by emotional individuals on internal states or exaggerating their symptoms.

Despite the similar predictive capacity of stress and emotional instability for chronic fatigue, the co-twin control analysis detected different influences by familial factors on the associations. Emotional instability was no longer associated with chronic fatigue when unmeasured genetic factors were controlled, whereas perceived stress was more strongly associated with the severer form of chronic fatigue (i.e., CFS-like illness) after the adjustment. The findings suggest that genetic factors mediate the entire association of emotional instability with chronic fatigue, and buffer or mask the impact of stress on the symptoms. In other words, the effect of stress on fatigue is likely to be external or exogenous, whereas that of emotional instability is probably internal or endogenous.

The considerable genetic mediation of the covariation between emotional instability and chronic fatigue clearly indicates biological mechanisms shared by the personality trait and the illness, rather than direct or phenotypic causality between them (Simonoff 2000). Recently, a study in SALT using the same longitudinal design (Kendler et al. 2006b) demonstrated the predictive power of emotional instability for new onset of major depression. In a trivariate twin analysis, the authors also found that the correlation between emotional instability and major depression was predominantly genetic. This is in line with the results in Study III (shown in Table 6), where the reduction in odds ratio was negligible when both zygositys were used and was substantial when only monozygotic twins were included. Furthermore, the other SALT study mentioned above (Charles et al., unpublished manuscript) also found that genetic factors were likely to mediate at least in part the prospective associations of emotional instability with chronic pain and related symptoms (although stress was not adjusted for). Taken together, these consistent findings probably suggest the existence of susceptibility genes in common to emotional instability (and its correlated psychiatric disorders) and functional somatic syndromes or symptoms.

There is no doubt that multiple genes are involved in the pathogenesis of comorbidity. Among others, potential candidates for Ac_1 that affect all of the six syndromes and disorders through L_1 are serotonin (5-hydroxytryptamine) transporter genes. Serotonin pathways are involved in a broad range of physiological and behavioral functions including both inhibition and facilitation of pain (Sommer 2006), and have links to mechanism in affective components of pain (Suzuki et al. 2004). Significant associations of serotonin transporter polymorphisms have been found with fibromyalgia (Bondy et al. 1999; Cohen et al. 2002; Offenbaecher et al.

1999), chronic fatigue syndrome (Narita et al. 2003), irritable bowel syndrome (Camilleri et al. 2002), and migraine (Juhász et al. 2003). In addition, it is well-recognized that the brain serotonin system plays a critical role in the regulation of mood and temperament (Lucki 1998), and therefore genetic variation in several key serotonin sub-systems is found to be associated with susceptibility to depression and anxiety (Caspi et al. 2003; Lesch et al. 1996; Pezawas et al. 2005). Given the modulatory effect of a variant of the serotonin transporter gene on amygdala reactivity to environmental threat (Hariri et al. 2005), the possible influences of serotonin on affective aspects of pain and affective or anxiety disorders may be exerted at least in part by way of amygdala and related limbic systems.

6.1.3 Possible mechanisms for the comorbidity of chronic pain

The second latent trait L_2 in Figure 3 loaded on the four functional somatic syndromes but neither of the two psychiatric disorders. As shown in Table 9, L_2 accounts for as much as 66% of the total variation in liability to chronic widespread pain. It is therefore reasonable to speculate that L_2 reflects etiological factors underlying chronic widespread pain to a large extent. Of the genetic variance estimated in the univariate analysis for women (54% in Study I and Table 3), it turned out that the latent genetic factor Ac_2 could account for more than half (29% of the total) in the multivariate analysis (Study IV and Table 9). Thus, familial aggregation observed in previous studies on fibromyalgia is likely due to genetic influences, primarily those of the latent trait L_2 .

Table 9 also shows the extent to which the traits L_1 and L_2 account for the total variance in other functional syndromes. The latent trait L_2 has less impact on the other three syndromes than on chronic widespread pain. In addition, the Ac_1 -to- Ac_2 ratio of genetic influences for chronic widespread pain was 1:5, whereas those for chronic fatigue, irritable bowel syndrome, and recurrent headache were nearly 1:1. This suggests that genetic influences on L_1 and that on L_2 are likely to be equally important for chronic fatigue, irritable bowel syndrome, and headache, whereas L_2 dominates the etiology of chronic widespread pain.

Results of the matched case-control and co-twin case-control analyses (Study II) shown in Table 4 indicated familial confounding in the associations between chronic widespread pain and the other functional syndromes. Greater familial confounding may be interpreted as greater effects of genetic factors in common in Figure 3 (i.e., Ac_1 and Ac_2) on the association through the latent traits. The high odds ratios that remained in the monozygotic only analysis (the right column of Table 4) reflect the importance of nonshared environmental effects Ec_1 and Ec_2 for the comorbidity. Although the associations between any two functional syndromes in Figure 3 are not quantifiable in terms of odds ratios, the results of parameter estimates are generally consistent with the conclusions that one can draw from Table 4.

Exaggerated pain is common in patients with fibromyalgia. Studies have consistently found that patients with fibromyalgia regarded sensory stimulation of healthy tissues as more painful (i.e., hyperalgesia) compared to not only pain-free controls (Price et al. 2002; Sorensen et al. 1998; Staud et al. 2001) but also other chronic pain patients (Julien et al. 2005). Although peripheral mechanisms can account for this, the lack of detectable peripheral tissue abnormalities and distinct spatial localization strongly supports the contribution of central mechanisms to the pain hypersensitivity (Staud & Smitherman 2002). Brain imaging by positron emission tomography (PET), single

photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI) also shows augmented pain experience of patients with fibromyalgia resulting in widespread activation of brain areas related to pain processing (Staud 2002). Abnormalities of central pain processing similar to fibromyalgia have also been reported in patients with irritable bowel syndrome (Verne & Price 2002) and recurrent headache (Okifuji et al. 1999). These findings converge on the notion that central sensitization due to abnormal central nervous system processing of noxious stimuli is a plausible pathophysiologic characteristic in common to functional somatic syndromes, which is represented as L_2 in the model.

In comparison with fibromyalgia and other pain-related syndromes, there is a dearth of evidence for central sensitization in chronic fatigue. It is possible that the relatively high loading from L_2 on chronic fatigue (33% of the total variation) merely reflects the overlapping symptoms in the criteria such as muscle or joint pain. In fact, 37% and 26% of fatigued subjects in this study reported muscle and joint pain, respectively (Sullivan et al. 2005b). However, there are a few studies suggesting central sensitization in chronic fatigue. Vecchiet et al. (1996) found lower pain thresholds at different sites in patient with chronic fatigue syndrome than in controls. Whiteside et al. (2004) reported a decrease of pain threshold in patients with chronic fatigue syndrome after graded exercise, whereas matched controls showed an increased pain threshold. Similar pattern was found in fibromyalgia patients (Vierck et al. 2001).

One may argue that central sensitization is influenced by two distinct, affective and physiological traits. Increased pain sensitivity can be due to anticipation of a painful stimulation or as a result of generalized psychological hypervigilance (McDermid et al. 1996). However, a study using stimuli in random order (Petzke et al. 2003a) demonstrated that enhanced pain sensitivity observed in fibromyalgia patients was not due to psychological influences on responding such as expectancies and hypervigilance. An implication of this is that any contribution of psychological components to altered pain sensitivity may be due to direct effects on nociceptive processing rather than to influences on pain reporting behavior (Fillingim 2003). In line with this finding, the present study found that L_1 and L_2 are independent of each other both genetically and environmentally. Thus, the mutual independence of L_1 and L_2 may suggest that the interplay between physiological and psychological aspects of pain occur at the level of manifest, phenotypic symptoms rather than at the level of latent, genetic or environmental factors.

In contrast to Ac_1 , it is more difficult to speculate on candidates for genetic influences represented as Ac_2 . One of only a few genes that have been identified to be associated with pain sensitivity in humans is catecholamine-O-methyltransferase (COMT) gene (Diatchenko et al. 2005; Zubieta et al. 2003). Associations of some COMT polymorphisms have been found with fibromyalgia (Gursoy et al. 2003) and migraine (Erdal et al. 2001). In a recent study using machine learning algorithms to analyze genetic data of patients with chronic fatigue syndrome (Goertzel et al. 2006), COMT gene was selected in the top genes with the highest importance. In addition, COMT has been suggested to have an impact in dopamine availability on prefrontal function and to be involved in susceptibility to schizophrenia (Egan et al. 2001). On the other hand, no consistent findings have been found in the association between COMT and depression or anxiety (Craddock et al. 2006). Thus, COMT is less likely to mediate the psychiatric comorbidity of functional somatic syndromes and is thereby more likely to be a candidate for L_2 than for L_1 . If so, it is not surprising that Gursoy et al.

(2003) did not find an association between COMT genes and psychiatric status in fibromyalgia cases.

6.1.4 Functional somatic syndromes: one *and* many

Overall, the two-component model clearly demonstrates that complex, multifactorial mechanisms underlie the etiology of functional somatic syndromes and their comorbidity. This model explains not only why the syndromes *co-occur* but also why they *differ*. That is to say, they co-occur because they share two latent traits, one of which has genetic influences in common to affectivity. And they differ because they are concurrently influenced by genetic and environmental factors specific to each of them. Thus, neither ‘lumpers’ nor ‘splitters’ are correct (Wessely & White 2004). In other words, lumpers are partly correct in the sense that the syndromes share part of the pathogenesis, whereas splitters are partly correct in the sense that the syndromes are not just different aspects of one illness. In addition, the two-component model convincingly denies the notion that these symptoms are psychogenic or ‘all in the mind’. Indeed, it is likely that the psychiatric comorbidity is due to shared genetic influences rather than secondary effects as a consequence of pain or fatigue; however, non-affective, physiological characteristics are at least as influential as the genetic influences on affectivity in the etiology.

Subjects with these syndromes are heterogeneous and can be categorized into subgroups. For example, Whitehead et al. (2002) posited the ‘dual-etiology’ hypothesis, saying that there may be a subgroup with a predominantly biological basis and another subgroup with a predominantly psychological basis, and that comorbidity is a marker for the latter subgroup. In fact, a SALT study based on the same sample as this thesis (Sullivan et al. 2005b) found heterogeneity in the subjects with chronic fatigue by using latent class analysis. It is therefore possible that the estimates for the impacts of the factors in the model differ when subgroups of subjects with fewer comorbid symptoms are used. However, as Whitehead et al. (2002) mentioned, the dual-etiology hypothesis is probably another example of an oversimplified view; the findings in Sullivan et al. (2005b) suggest that the distribution of comorbid symptoms is not dualistic but like a spectrum without a cut-off point. In addition, given that the main objective of the present study is to understand the comorbidity of functional somatic syndromes, it would be meaningless to exclude subjects with multiple symptoms.

In essence, the view proposed in this thesis is in line with a ‘paradigm shift’ suggested by Sharpe & Carson (2001). That is, the etiological model of unexplained somatic symptoms (or functional somatic syndromes) should “acknowledge the neurobiological correlates of the patients’ somatic symptoms as well as the influence of psychological factors” (pp. 929). Thus, it is necessary “not only to continue exploring the psychological mechanisms that produce symptoms but also to integrate these studies with biological investigations” (pp. 929). The two-component model supports psychotherapeutic effect of medical management, e.g., antidepressants and cognitive-behavioral techniques. Furthermore, this model proposes a new agenda for future research. For example, in the search for susceptibility genes, researchers should be aware of which genetic factor in the etiological pathways they are focusing on. Different strategies will be required when searching one of the common genetic factors Ac_1 and when searching one of the specific genetic factors, e.g., As_6 . On the

other hand, specific environmental factors (Es) play key roles in differentiating these syndromes. This probably reflects the fact that patients with each syndrome have different triggering experiences, such as whiplash or muscle injury for chronic widespread pain and viral infection for chronic fatigue. Identifying such precipitating or exacerbating factors specific to each syndrome will not only facilitate the understanding of the etiology but will also provide some clues to prevent the syndromes from developing after such triggering experience. It is hoped that the model presented here will serve as a road map to help researchers navigate while exploring the labyrinth of as yet unresolved functional somatic syndromes.

6.2 METHODOLOGICAL ISSUES TO BE CONSIDERED

The present study is solely based on sub-cohorts of the Swedish Twin Registry. In this section, general issues concerning epidemiological studies in general are first discussed; then issues specific to the twin design are considered.

6.2.1 Assessment procedures

All the screening data used in this study (except the exclusion criteria for chronic fatigue) were obtained from a telephone interview. The exclusion criteria for chronic fatigue were based on medical record review by physicians. In epidemiological studies with large, community-based samples, it is unfeasible or unrealistic that all participants undergo medical examination. Thus, inferences should be tempered when comparing the present findings with those of studies based on clinical evaluation. However, questionnaire- or interview-based screening for chronic pain has been suggested as being useful in general population surveys. White et al. (1999b) reported relatively high positive predictive value (70.6%) and test-retest reliability (81.0%) using four items on pain and two items on fatigue.

Of the four functional somatic syndromes and the two psychiatric disorders, the symptoms should be present at the time of interview for chronic widespread pain and chronic fatigue, whereas lifetime occurrence was used for irritable bowel syndrome, recurrent headache, major depression, and generalized anxiety disorder. Thus, the term *comorbidity* in this study may need to be considered as a broad sense, i.e., the lifetime comorbidity.

6.2.2 Reliability

Reliability is the degree to which a measurement is reproducible, e.g., on different occasions. For binary (dichotomous) data, reliability can be estimated directly using Cohen's k (Cohen 1960). To evaluate test-retest reliability of the SALT interview, 105 interviewees were selected at random and re-contacted for a second interview two weeks later. Cohen's k coefficients for general muscular pain lasting ≥ 3 months and for prolonged fatigue lasting ≥ 6 months were 0.85 and 0.76, respectively. Thus, the screening interview can be considered to have good to excellent reliability for these measures.

6.2.3 Internal validity

Internal validity refers to the extent to which a relationship found to be statistically significant is a causal relationship. Other than causality, there are three possibilities to account for an observed relationship: bias, confounding, and chance.

6.2.3.1 Selection bias

The case-control study design is prone to selection bias. Controls should be representative of the population from which cases arise in terms of their exposure distribution. Because the present study was based on a national sample from the twin registry covering the whole of Sweden, the threat to the assumption that cases and controls came from the same population source should be minimal. When several illnesses are included in a study, it is appropriate to use population-based samples; as first described by Berkson (1946), spuriously high comorbidity is a well-known limitation of hospital-based case-control studies.

If those who did not participate in the study were systematically different from participants, selection bias may come into play. Predictors of participation in the SALT interview were being a monozygotic twin, fewer hospitalizations, having a co-twin who was also eligible for the study, female gender, and age. Although it is unlikely that a particular illness examined in this study systematically distorted the participation, the respondents to the interview may be somewhat different from the general population. However, the significant mean differences in participation were largely due to the large sample size rather than of meaningful significance.

In longitudinal studies, attrition is another issue of concern. If those who dropped from the study did so due to factors relevant to the exposure or outcome of interest, the observed relationship can be distorted. The proportion of subjects who responded to the questionnaire in 1972-73, were alive but did not participate in SALT in 1998-2002 was less than 10%. Thus, selection bias due to attrition can be considered minimum.

6.2.3.2 Information bias

The information used in this study was based on self-report, collected through either the telephone interview or mailed questionnaire. Information bias can occur when interviewers collect information from cases in more detail than controls (interviewer bias) or when cases give information with more accuracy than do controls (recall bias).

Misclassification of a subject's exposure or outcome status is another form of information bias. For dichotomous exposures and outcomes, the direction of bias in the estimate of relative risk will be toward the null if misclassification is nondifferential. On the other hand, if misclassification occurs differentially for the comparison groups, the direction of bias can be in any direction. In this study, the results would be affected if misclassification of illnesses occurs differently in monozygotic twins and dizygotic twins. However, it is unlikely that the extent of possible misclassification differs across zygosity. Thus, the observed heritability is not likely overestimated.

6.2.3.3 Confounding

Confounding occurs when the study samples in the comparison groups are imbalanced with respect to other characteristics that are independent of the outcome under study. A confounder is associated with both the exposure and outcome, but not an intermediate factor in the pathway between them. Two of the most common confounders, age and sex, were adjusted for throughout this study. In Study III, personality traits (emotional instability and extraversion) and perceived stress were mutually adjusted because they were correlated. Possible residual confounders may be socioeconomic status, education, or undetected health conditions. For example, subjects who had heavy workloads might have reported stress and developed fatigue later. However, chronic fatigue is such an illness with virtually no established risk factor that it would be incorrect to adjust for potential intermediate factors in its causal pathway.

6.2.3.4 Random error

Random errors are errors that may give a deviation from the true value in a study. Random errors can be minimized if a sufficiently large sample is used or the study is repeated a sufficiently large number of times. Confidence intervals (CI) reflect to what extent the estimate is likely obtained due to chance. In Table 3, the CI of heritability for chronic widespread pain in men included null. However, the patterns of intrapair similarity shown in Table 2 suggest that this estimate may be true rather than attributable to chance.

Random measurement error is another source of chance findings. In classical twin modeling, measurement error is included in the estimate of nonshared environmental effects. As mentioned before, the indicators of reliability are high for chronic widespread pain and chronic fatigue; however, it is possible that measurement error is illness-specific and the proportion of measurement error in specific environmental effects (E_s) differs from illness to illness. Thus, care should be taken when comparing E_s estimates between or across illnesses in the multivariate analysis.

6.2.4 External validity

External validity is the extent to which a finding is generalizable to and across persons, time periods, and settings. Generalizability across persons refers to whether the finding can be applied to other populations with different age distribution, sex, culture, etc. The following three aspects may be of particular note.

6.2.4.1 *To younger populations*

First, the age of this sample was over 40, and under 65 in Study III and IV. Because the prevalence rates for chronic widespread pain and chronic fatigue are highest in middle age, it is likely that the majority of subjects had passed the window of onset of these illnesses at the time of the interview. Although it is hard to speculate whether the results are generalizable to younger populations, Mikkelsen et al. (2001) implies that widespread pain in children might be different from that in adults. The authors

did not find sex difference in prevalence in their sample. On the other hand, studies on chronic fatigue in children and adolescents (Farmer et al. 2004; ter Wolbeek et al. 2006) reported prevalence (1.9% in the UK), sex difference, and comorbid symptoms that are similar to those in adults. It will be of interest to conduct longitudinal research at pediatric settings in order to examine risk factors for the development of chronic fatigue.

6.2.4.2 To the other sex

Second, the results of Study IV were based on women only, due to a lack of statistical power in men. Sullivan et al. (2005a) as well as Study I suggest that the relative importance of genetic and environmental influences on chronic widespread pain and chronic fatigue does not differ in men and women. However, using the same SALT sample including opposite-sex twins, Kendler et al. (2006a) found sex differences in the relative importance of genetic and environmental influences on major depression. Furthermore, the genetic correlation was less than one, i.e., not all genes for major depression are expressed the same in men and women. Although there has been no evidence to suggest different genetic factors influence emotional instability (Bouchard & McGue 2003), the role of personality in determining the way to cope with stress can be sex-specific (Kato & Pedersen 2005). Thus, it may be too early to tell whether the results of Study IV are generalizable to men.

6.2.4.3 To non-twin populations

Finally, the participants were all twins. If twins are different from singletons (non-twins) in terms of the outcome of interest, the results will not be applicable to the general population. The prevalence for chronic widespread pain in this study was 4.1%, which is comparable to another Swedish study (4.2%; Lindell et al. 2000). The prevalence did not differ across zygosity, which ensures that there is no association between the illness and type of zygote. However, the variance was statistically higher in monozygotic twins than in dizygotic twins. This does not affect the generalizability but could affect the goodness-of-fit of the models. In addition to generalizability, twin studies require considerations specific to this design, which are discussed in the next section.

6.2.5 Issues of twin studies

6.2.5.1 Zygosity determination

As mentioned in Section 3.1.4, zygosity was determined by self-reported physical similarity, with an accuracy of 98% (Lichtenstein et al. 2002). If misclassification occurs in zygosity determination, it would affect the results. However, similar to the discussion regarding information bias in Section 6.2.3, it is likely that misclassification of zygosity, if any, is nondifferential in cases and controls.

6.2.5.2 Nonadditive genetic effects

In both univariate and multivariate analyses of this study, only additive genetic effects were taken into account as the source of genetic variance. As shown in Table

2, the tetrachoric correlations did not strongly suggest nonadditive genetic effects, particularly in women. If there had been significant nonadditive genetic variation and this was not accounted for the models, the results would have been an overestimation of heritability.

6.2.5.3 Assumptions in the twin analysis

Twin studies are based on some assumptions such as equal environment assumption, assortative mating, and gene-environment interaction and correlation.

First, it is assumed that the extent to which monozygotic and dizygotic twin pairs share family environment (including the prenatal one) is the same. This is reflected in the environmental correlation within pairs, which was set to be 1.0 for both monozygotic and dizygotic twins. If the similarity in trait-relevant family environmental influences on the outcome of interest is greater in monozygotic twins than that in dizygotic twins, the heritability for the outcome will be overestimated. Although it is unknown whether this assumption is valid for functional somatic syndromes, studies of psychiatric disorders, personality, intelligence, etc. have found little or no indication of its violation (Kendler et al. 1993; Scarr & Carter-Saltzman 1979).

Second, it is also assumed that mating occurs at random. When mating occurs based on similarity in a particular trait, which is called assortative mating, shared environmental effects may be overestimated. Although no data are available for functional somatic syndromes, there is no reason to believe that this is the case.

Finally, it is possible that genetic susceptibility to a certain disease differs depending on the environment, i.e., gene-environment interaction. Although it is thought that the interaction effects are generally small, this could be a source of bias. Recently, a novel approach has been developed as an extension of the 'ACE' model (Purcell 2002), which can be useful if a priori knowledge about interactions exists and specific environmental factors are measured. By using this technique, Yamagata et al. (2006) found that the heritabilities for depression and anxiety decreased as the subjects reported more stressful events. It would therefore be of interest to extend the present study to investigations of possible gene-environment interactions in functional somatic syndromes.

6.3 WHAT DO WE STILL NEED TO KNOW?

As mentioned in Section 6.1.4, the present study paves the way for future endeavors in exploring functional somatic syndromes. An important question not focused on in this study is the reason for sex difference in prevalence. Finding no sex difference in type or magnitude of genetic influences on chronic widespread pain may suggest that the causes lie in somewhere outside the pain pathways such as adaptability to stressful symptoms, or reflect reactions to environmental exposures such as stressful environment. Interestingly, when an additional question about self-reported tender or sore spots was included in the case definition of chronic widespread pain, the magnitude of genetic influences showed significant sex difference. Several population-

based studies have found high correlations between tender point counts and the level of distress (Croft et al. 1994; Wolfe 1997), and a higher likelihood of having tender points in women than in men (Wolfe et al. 1995a). Thus, sex differences in genetic influences on affectivity may account for the sex differences in experiencing or reporting tenderness in their body, resulting in higher female predominance in fibromyalgia than in chronic widespread pain (Petzke et al. 2003b). Another explanation for the higher prevalence among women might be dysfunctions of endogenous or exogenous estrogen and other endocrines.

As also mentioned previously, the subjects may be heterogeneous (Sullivan et al. 2005b). It is therefore of interest to use statistical techniques such as latent class analysis. Genetic analyses of latent classes may reveal subgroups that are more heritable than others. Finding subgroups can be particularly useful in clinical settings, in that it may enable physicians to diagnose patients more precisely and to offer them more effective and appropriate treatment.

Detecting genetic influences in functional somatic syndromes should encourage researchers to move on to the stage of gene hunting. The above-mentioned heterogeneity study as well as the two-component model presented in this study will help to identify candidate genes and target individuals so that more reliable and reproducible results can be obtained with increased statistical power. Providing evidence for greater genetic influences on certain intermediate traits than on others will also help researchers to identify biological components in the pathological pathways.

Environmental factors specific to each syndrome also deserve further investigation. Longitudinal studies using monozygotic twins discordant for environmental exposures (e.g., injury, infection, stressful events in childhood, etc.) would contribute greatly to understanding the role of these exposures, although enormous samples and long-lasting follow-ups are required.

Finally, it would be desirable to share new knowledge about the syndromes with those who are suffering from them. The controversy over functional somatic syndromes involves not only researchers and physicians but also patients with these syndromes (Barsky & Borus 1999). Disease attributions to incorrect causality can lead to refractoriness of the symptoms, malfunction of physician-patient relationship, and perpetuation of disabilities. It is therefore essential to integrate biological and psychosocial aspects of these illnesses to both better understanding of the etiology and more effective management.

7 CONCLUSIONS

1. Individual differences in liability to chronic widespread pain reflect modest genetic influences. The type and magnitude of these influences do not differ significantly across sex.
2. The comorbidity between chronic widespread pain and related illnesses is mediated by familial influences to a varying degree. It is likely that these influences are exerted mainly through central sensitization.
3. The comorbidity between functional somatic syndromes and psychiatric disorders is probably due to genetic predisposition to affective personality. Daily stress is a direct risk factor for chronic fatigue, impacting beyond the influences of familial factors.
4. Functional somatic syndromes are attributed to latent etiological components (one of which is affective and the other not) that are shared by these syndromes. The differences among the syndromes are derived primarily from environmental factors specific to each.

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