Karolinska Institutet

Institutionen för kliniska vetenskaper, Danderyds sjukhus, Enheten för obstetrik och gynekologi

PROVOKED VESTIBULODYNIA – STUDIES ON PAIN GENETICS AND PAIN CO-MORBIDITY

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Ulrika Heddini

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1 ABSTRACT

Objective: The purpose of this thesis was to investigate a possible genetic predisposition for developing provoked vestibulodynia (PVD), focusing on previously defined single nucleotide polymorphisms (SNPs) in three genes with a known influence on endogenous pain modulation: *GCH1*, *OPRM1* and *5HT-2A*. We also investigated the effects of any potential interactions between these SNPs and the use of hormonal contraceptives, serum levels of β-endorphin and symptoms of anxiety and depression, on the risk of developing PVD and general pain sensitivity. Potential predictors of treatment outcome and the prevalence of pain co-morbidity among women with PVD were also explored.

Materials and methods: The thesis is based on one descriptive study and three case-control studies which included 109 women with PVD and 103 healthy controls who underwent quantitative sensory testing and filled out study-specific questionnaires. Venous blood samples were collected for genetic analyses and β-endorphin quantification.

Results: The results showed that the probability of being diagnosed with PVD was elevated in carriers of the 118A genotype (rs1799971) of the *OPRM1* gene (OR 1.8) and the 102C genotype (rs6313) of the *5HT-2A* gene (OR 2.9) but not in carriers of the studied SNPs in the *GCH1* gene (rs8007267, rs3783641 and rs10483639). However, there appeared to be an interactive effect between the *GCH1* SNPs and use of hormonal contraceptives, with respect to pain sensitivity among women who were currently receiving treatment for PVD. There was increased pressure pain sensitivity among participants carrying the 118A genotype of the *OPRM1* gene and those with PVD were more sensitive than healthy controls to pressure pain and had higher levels of plasma β-endorphin. The probability for PVD was also elevated among participants with symptoms of anxiety (OR 5.2). Higher prevalence of concomitant bodily pain was correlated with the 102C genotype of the *5HT-2A* gene and with anxiety. A successful treatment outcome was more likely in women with PVD who had fewer other concomitant pain conditions and in those with secondary PVD. The number of other bodily pain conditions was also associated with the intensity of coital pain.

Conclusions: The results of these studies indicate that specific genetic polymorphisms in the opioid and serotonin systems that affect endogenous pain modulation contribute to the risk of developing PVD. This substantiates the findings of earlier studies, which found greater general pain sensitivity and more anxiety symptoms in patients than in controls. Women with PVD who had more pronounced general pain dysfunction and those who had primary PVD were less likely to achieve a satisfactory treatment outcome. These findings strengthen the concept that PVD is a general pain condition.

Clinical implications: It is proposed that a careful medical history be carried out in women with PVD to investigate the degree of concomitant pain disorders and to establish the subgroup of PVD so as to identify patients who could benefit from referral to specialist centers. Early recognition and treatment of the disorder could, in addition to restoring the sexual health of the affected women, also prevent aggravated chronic pain problems in this patient group.

Keywords: provoked vestibulodynia, dyspareunia, chronic pain, general pain, GCH1, OPRM1, 5HT-2A, genetic polymorphism, co-morbidity, anxiety, depression

2 LIST OF PUBLICATIONS

This thesis is based on the studies reported in the following original papers, which will be referred to by their Roman numerals (I - IV).

I. Ulrika Heddini, Nina Bohm-Starke, Kent W. Nilsson, and Ulrika Johannesson. **Provoked Vestibulodynia – medical factors and co-morbidity associated to treatment outcome.** J Sex Med 2012;9:1400–1406.

II. Ulrika Heddini, Nina Bohm-Starke, Alfhild Grönbladh, Fred Nyberg, Kent W. Nilsson, Ulrika Johannesson. **GCH1-polymorphism and pain sensitivity among women with provoked vestibulodynia.** Mol Pain 2012; 12:8:68.

III. Ulrika Heddini, Ulrika Johannesson, Alfhild Grönbladh, Fred Nyberg, Kent W. Nilsson, Nina Bohm-Starke. **A118G polymorphism in the μ-opioid receptor gene and levels of β-endorphin are associated with provoked vestibulodynia and pressure pain sensitivity.** Scan J of Pain 2013 Published online; http://dx.doi.org/10.1016/j.sjpain.2013.10.004.

IV. Ulrika Heddini, Nina Bohm-Starke, Alfhild Grönbladh, Fred Nyberg, Kent W. Nilsson, Ulrika Johannesson. **Serotonin receptor gene (5HT-2A) polymorphism and anxiety are associated to provoked vestibulodynia and co-morbid symptoms of pain.** Submitted

To my family,
and in memory of my mother

3 TABLE OF CONTENTS

4 LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
AR	Androgen receptor
AVPR1A	Arginine vasopressor 1A (receptor gene)
BH4	6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin
CBT	Cognitive behavioral therapy
CGRP	Calcitonin gene-related protein
COC	Combined oral contraceptive
CPM	Conditioned pain modulation
CSF	Cerebrospinal fluid
CWP	Chronic wide-spread pain
DNIC	Diffuse noxious inhibitory control
EDTA	Ethylene diamine tetra-acetic acid
ER	Estrogen receptor
GABA	Gamma-aminobutyric acid
GCH1	Guanisine triphosphate cyclohydrolase 1
GLM	General linear regression model
HADS	Hospital Anxiety and Depression Scale
HC	Hormonal contraceptive
GWS	Genome-wide screening
IBS	Irritable bowel syndrome
IL	Interleukin
ISSVD	International Society for the Study of Vulvovaginal Disease
MRI	Magnetic resonance imaging
NALP3	Nucleotide binding oligomerization domain-like receptor
NMDA	N-methyl d-aspartate
NS	Nociceptive-specific neurons
OPRM1	Opioid receptor molecule 1 (μ-opioid receptor)
POMC	Pro-opiomelanocortin
PPT	Pressure pain threshold
PRA and PRB	Progesterone receptor A and B
PVD	Provoked vestibulodynia
RIA	Radioimmunoassay
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
TMD	Temporomandibular pain disorder
TNF	Tumor necrosis factor
VAS	Visual analog scale
WDR	Wide dynamic range neurons
5HT-2A	5-Hydroxytryptophan-2A (serotonin receptor)

5 INTRODUCTION

Creta Kano´s long story – An inquiry into the Nature of Pain

"And when I say `pain´ that is exactly what I mean. …Plain, ordinary, direct physical – and for that reason, all the more intense – pain: headache, toothache, period pains, lower back pain, stiff shoulders…. All my life I have experienced physical pain with far greater frequency and intensity than other people. ….. In college, I found a boyfriend, and in the summer of my first year I lost my virginity. Even this – as I could have predicted – gave me only pain…. Whenever I slept with him, the pain would bring tears to my eyes."

From 'The Wind-up Bird Chronicle' by Haruki Murakami

5.1 PROVOKED VESTIBULODYNIA

5.1.1 Introduction and historical summary

Dyspareunia is a common health problem. The most common type of dyspareunia among premenopausal women is provoked vestibulodynia (PVD). Medical records more than a century ago described a condition characterised by "hyperaestesia of the vulva" with "occasional red spots" [1, 2]. In the late 1970s and early 1980s, studies described "chronic inflammation of the posterior vestibular mucosa", "infection of the minor vestibular glands", and "focal vulvitis" with symptoms and signs very similar to those we currently associate with PVD [3-5]. In 1983 the first patients were treated with surgical perineoplasty. The term vulvar vestibulitis syndrome was proposed by Friedrich in 1987 and was widely used for many years. Friedrich also stipulated diagnostic criteria, which are still used but sometimes modified [6]. To harmonize these criteria with the classification of other chronic pain disorders and to give a more accurate description of the condition, the terms PVD and localized provoked vulvodynia (LPV) were suggested by the International Society for the Study of Vulvovaginal Desease (ISSVD) in 2003 and these are currently the standard terms [7].

5.2 PREVALENCE AND DIAGNOSIS

The clinical diagnosis of PVD is one of exclusion. The condition is characterized by pain upon light touch, pressure and stretch of the tissue around the vaginal opening, with no spontaneously ongoing pain. The diagnostic criteria are: long-standing entry dyspareunia (minimum duration of 6 months), tenderness to light touch such as cotton swab palpation (the Q-tip test), and absence of infection or other gynecological or dermatological disease [6, 8]. It has been difficult to establish the prevalence of PVD since not all affected women will seek medical attention and a correct diagnosis is not always made upon examination. However, several studies have estimated the prevalence as 10-15% [9-11].

Two sub-categories of PVD have been identified: primary PVD, where pain occurs at the first attempt of vaginal entry (intercourse or tampon use); and secondary PVD, where pain occurs

after a period of normal function [12-14]. The sexual dysfunction and common inability to engage in vaginal intercourse caused by the condition have a well documented, severe, negative impact on the quality of life of the affected women and their partners [15-19].

5.2.1 Etiology

Clinical and research interest in female sexual health, including PVD, has increased during recent decades. Nonetheless, the etiology of PVD remains to a considerable extent unclear. Studies have shown evidence of patho-physiological changes in three interdependent systems: the vestibular tissue, the pelvic floor muscles, and the pain regulatory pathways of the central nervous system [20]. The etiology is currently considered to be multifactorial [21, 22]. However, there is still some controversy regarding whether PVD is predominantly an organic or a functional disorder. There is scientific evidence to support both hypotheses. The first hypothesis suggests the presence of neurogenic inflammatory pain induced by a trigger such as, for example, recurrent candida infections or hormonal effects, resulting in long-standing pain in susceptible individuals with dysregulation of endogenous pain signalling [23-28]. The findings include neural hyperplasia, increased inflammatory mast cell infiltrates, and peripheral sensitization of the vestibular mucosa, which support the idea of neurogenic inflammation and contradict the theory that psychological factors are the sole cause of pain [29-34] . Furthermore, there is augmenting support for PVD being part of a general pain hypersensitivity disorder [11] with an enhanced systemic pain response [35, 36]. Women with PVD have more painful tender points and a higher sensitivity to experimental pain stimuli in non-genital regions than unaffected women [33, 37, 38]. There are associations between PVD and conditions such as fibromyalgia, painful bladder syndrome, temporomandibular pain disorder (TMD), chronic wide-spread pain (CWP), chronic fatigue syndrome and irritable bowel syndrome (IBS) [20, 39, 40]. Magnetic resonance imaging (MRI) reveals that the brain regions activated during painful vestibular contact in women with PVD are the same as those activated in patients with fibromyalgia, IBS and neuropathic pain [41, 42].

The other hypothesis suggests that PVD is largely psychosomatic in nature, with pain elicited by physical contact triggering a vicious cycle of hypervigilance, anxiety, and pelvic floor muscle hypertension leading to increased pain [43]. Several studies have reported that psychological traits and disorders such as low levels of pain self efficacy, elevated harm avoidance, high tendency of catastrophizingv, anxiety and depression [44-47] are more common in PVD patients than in healthy controls. However, it remains unknown whether the psychological traits and sexual dysfunction described are an antecedent to the development of PVD or whether the psychosexual problems and pelvic muscle dysfunction appear as a result of the long-standing vestibular pain.

Clinical experience suggests that PVD patients display different patterns that neither theory can explain sufficiently on its own and a combination of the two, involving both biomedical and psychosexual causes, is probably the most likely scenario (see Figure 1). Moreover, several studies show different characteristics for primary and secondary PVD, and there are speculations that these subgroups could have different etiologies [34, 48-50].

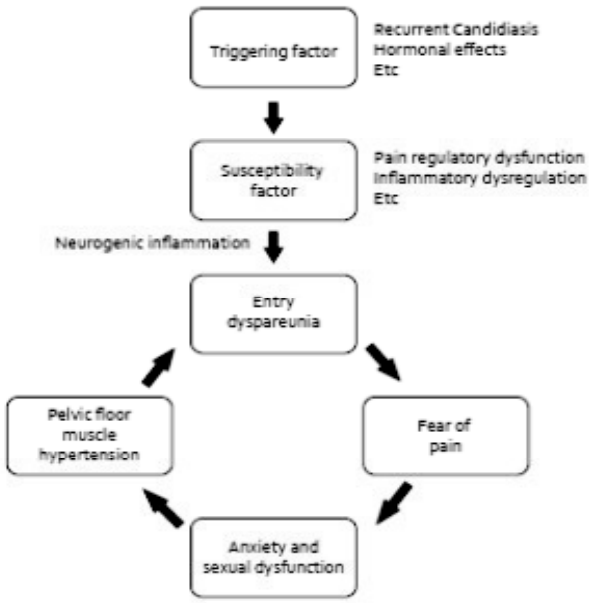


Figure 1. Factors involved in initiating and maintaining the vestibular pain in PVD.

5.2.2 Genetic background

A familial aggregation for PVD has not yet been proved, but a familial aggregation for other chronic pain syndromes associated with PVD, such as fibromyalgia, migraine and IBS, has been reported [51-53]. Genes associated with these disorders include those encoding catechol-O-methyl transferase and the serotonin (5-hydroxytryptophan) receptor 5HT-2A [54].

The assumed genetic predisposition for developing PVD has been investigated to some extent. As described above there are scientific evidence of an ongoing neurogenic inflammation in the vestibular mucosa in women with PVD and there are findings offering a genetic support to this concept, including PVD-associated polymorphisms in genes affecting the pro-inflammatory immune response, with correlations to genetic variants involved in the regulation of this response and less potent anti-inflammatory counterparts [55, 56]. For instance, a higher presence of a specific allele of the gene coding for the IL-1 receptor antagonist protein was found among women with PVD [57]. Previous studies have reported an association between that allele and a number of inflammatory diseases in which IL-1 was implicated in the inflammatory mechanism [58]. Furthermore, Foster and co-workers reported that PVD patients are more likely to be homozygous for allele 2 of the IL-1 receptor antagonist gene and to carry at least one of six loss-of-function polymorphisms in the melocortin-1 receptor gene. The effect of both of these polymorphisms combined was additive for the risk of developing PVD [59]. An additional study on the IL-1 system showed that allele 2 in the IL-1β gene appeared to be more common in women with PVD than in healthy women, which suggests that susceptibility to the PVD syndrome might be higher in individuals carrying this polymorphism [60].

Moreover, recurrent vulvo-vaginal Candida infections have been reported as a trigger of PVD symptoms in some women, a phenomenon that might be explained by additional genetic differences [23, 25]. A higher frequency of a variant of the gene coding for mannose-binding lectin, an innate immune antimicrobial protein that inhibits Candida proliferation, has been associated with PVD [61]. Additionally, a polymorphism in the inflammasome NALP3 gene (CIAS1), which codes for a macromolecule that regulates the release of IL resulting in reduced production of active IL-1β, has also been reported in PVD patients [62]. IL-1β is necessary for the recruitment of the phagocytes that inactivate yeasts and lower levels might result in a less effective immune response to infection.

5.2.3 Treatment alternatives and treatment outcomes

There is no standardized treatment for PVD; treatment options, if available, are empiric and differ between care providers. Management is often long-standing and outcomes vary. Very few randomized, placebo-controlled trials have been performed and the level of evidence is generally low [63-66]. This absence of consensus on treatment can be explained by a lack of knowledge. While surgery was the predominant treatment in the 1990s, less invasive treatment modalities are now usually tried first. The state of the art of vulvodynia management developed by the ISSVD is described in 'The Vulvodynia Guideline', which proposes a multi-disciplinary treatment approach using a combination of pain management, pelvic floor muscle rehabilitation and psychosexual counseling, including cognitive behavioral therapy (CBT), as the main alternatives to surgery [67]. A short compilation of the most common treatment alternatives is shown in table 1.

Table 1. PVD treatment alternatives. A multi-disciplinary combination of several modalities is often recommended.

Common treatment alternatives	
Pain management	Lidocaine gel 2-5% , local desensitization with topical applications 3-5 times/day or overnight
	Topical ointment
	Amitryptiline 30 - 50 mg x 1, orally, ≥ 2 months
	Gabapentin 300- 600 mg x3, po, ≥ 2 months
Pelvic floor rehabilitation	Physiotherapy and/or electromyographic (EMG) biofeedback
	Botulinum toxin A injection of 20-25E in the bulbocavernosus muscle bilaterally, 1 x 2-3
Surgery	Posterior vestibulectomy
Psychosocial counseling	Cognitive behavioral therapy / sexology counseling

Attempts have been made to evaluate treatment outcomes. The primary outcome in most trials is reduction in coital pain, measured by patient self-ratings on a numeric scale, subjective definitions of improvement focusing on the functional aspect, or measurements using vulvar algesiometers based on examiner provocation [68]. Other outcome measures used in previous studies include patient-rated improvement in quality of life and/or sexual function [69]. In the literature, success rates for vestibulectomy range from 60% to 90% versus 40% to 80% for non-surgical interventions [70-76]. The treatment outcome could be compared to an improvement of 30% to 40% in patients treated with placebo [77, 78]. However, there is no consensus regarding the definition of a successful treatment outcome, and methods for evaluation of outcomes as well as follow-up time vary between studies [64, 65, 79-81] . Recently the tampon test, where pain on insertion and removal of a vaginal tampon is rated from 0 to 10, has been shown to be a feasible way of determining PVD treatment outcomes. This evaluation method offers the advantage of allowing for the inclusion of study participants who are not currently engaging in vaginal intercourse [82].

A limited number of studies investigating predictors of treatment outcome have been published. Lower levels of anxiety and catastrophizing and higher levels of pain self efficacy prior to treatment appear to be associated with reduced coital pain and improved sexual function. Moreover, a diagnosis of secondary PVD and fewer concomitant pain conditions such as headache, IBS, and back pain have also been linked to a better response to treatment [75, 83-85].

5.3 THE VULVAR VESTIBULE

5.3.1 Anatomy

The vulvar vestibule is defined as the part of the vulva surrounding the vaginal opening. The anterior anatomical border of the vestibule is the frenulum of the clitoris and the posterior border is the mucocutaneous border of the perineum. The lateral borders extend from the hymenal ring to the so-called Hart´s line on the inner aspect of the labia minora. The Hart´s line represents the junction between the inner squamous cell epithelium and the keratinized epithelium of the labia. There are several glands in the vestibulum; the main glands are the Bartholin´s glands which are located in the posterior part, beneath the bulbocavernosus muscle, with duct openings located close to the hymenal ring at approximately 4 and 8 o´clock and the Skene´s glands located para-urethrally with duct openings close to or entering the urethral orifice. A schematic presentation of the vestibulum is shown in Figure 2.

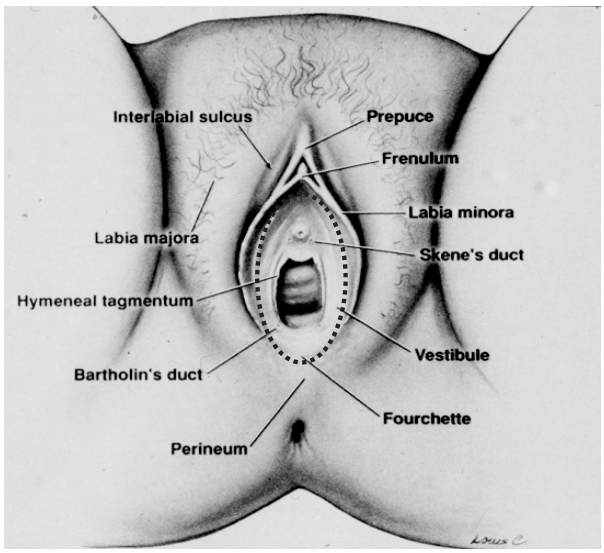


Figure 2. The vulvar vestibule; indicated by the dotted line.

5.3.2 Histology

The vestibulum is derived from embryonic endoderm and the skin-bearing parts of the vulva are of ectodermal origin. The clitoris and the labia majora originate from the anterior genital folds, whereas the urethra, the vagina and the vestibular glands are formed from the urogenital sinus. The vestibular epithelium resembles those of the vagina and the mouth; it is non-pigmented and covered by a thin keratinized layer [86]. The epithelium contains superficial large, flattened cells containing glycogen; pycnotic nuclei are frequent. Immune cells such as Langerhans cells, which present antigens to circulating T-cells and lymphocytes, are also present. Like the buccal mucosa, the vestibular mucosa has an increased permeability to external penetrants [87]. There is a dynamic junction between the epithelium and the underlying connective tissue, with dermal papillae projecting up into the epithelium to create a wrinkled profile [88]. The underlying connective tissue features collagen fibers and capillaries, and arterioles and venules are found below the lamina propria. The arterial blood flow is derived from the internal iliac and femoral arteries and the venous drainage occurs via the corresponding veins.

5.3.3 Hormonal receptors and effects

The effects of sex hormones on the endometrium and vaginal mucosa [89, 90] are well known, but the steroid receptors in the vestibular mucosa have only recently been investigated. The two estrogen receptors, ERα and ERβ, are both present. ERα-expressing cells are predominately distributed along the basal membrane and are seen less frequently in stromal and vascular endothelial cells. ERβ-positive cells have similar distribution patterns, but are more abundant in the stromal and vascular endothelial cells. ER levels appear to remain stable throughout the menstrual cycle in healthy women. However, in the follicular phase, total ERα levels are higher in women with PVD than in healthy controls. There are no differences in expression of ERβ between these groups [91].

Cells expressing progesterone receptors PRA and PRB are sparse, with no differences between PVD patients and healthy women. Cells expressing the androgen receptor AR are found in the suprabasal part of the epithelium and in the stroma, and glucocorticoid receptors are found in most cells in the stromal tissue, including vascular endothelial cells, with no differences between healthy women and those with PVD [92].

The use of combined oral contraceptives (COCs) has been identified as a risk factor for PVD [25, 26, 88] and a subgroup of PVD patients improves after cessation of COC use. There are some possible explanations for this observation. The morphology of the vestibular mucosa is altered during the luteal phase of the menstrual cycle when dermal papillae are sparser. This situation is seen in a more constant fashion among users of COCs where the papillae are both sparser and lower [88]. Furthermore, users of COCs had lower punctuate mechanical pain thresholds in the vestibulum than non-users in one study [93]. This suggests an effect of sex hormones, most likely the progestins, on the vestibular mucosa, possibly making it more vulnerable to mechanical strain and more sensitive to pain. Moreover, the secretion of mucous from the main glands in the vestibulum is thought to be androgen-dependent [94], and decreased lubrication associated with COC use has been described [95].

5.3.4 Innervation

The vestibulum is innervated by the pudendal nerve, which originates from the sacral nerve roots S2-S4. Although the vestibule is by definition visceral tissue, it is considered to have non-visceral innervation with sensations similar to those evoked in the skin [96]. The external genital area is supplied with both myelinated and unmyelinated nerves, terminating in various endings involved in the perception of touch, pressure and pain [97]. Unevenly distributed intra-epithelial free nerve endings have been found in both women with PVD and healthy controls; however, the number of intra-epithelial free nerve endings was significantly higher in women with PVD [30]. These nerve fibers were of sensory origin and were immuno-positive for calcitonin gene-related protein (CGRP), thus possibly contributing to a neurogenic inflammation in the tissue when activated [31].

5.4 PERIPHERAL AND CENTRAL PAIN MECHANISMS

Pain is a very complex phenomenon. The normal function of pain is to alert the individual to potential tissue damage; this is known as inflammatory or nociceptive pain. When the noxious stimulus is removed, the pain remits. However, in some cases a patho-physiological state can emerge resulting in persistent pain without any biological advantage, often paroxysmal, and independent of stimuli. Long-standing pain originating from damage to or diseases of the peripheral nerves or the central nervous system is called neuropathic pain. When no obvious nerve damage or other explanations for the pain can be found, it is called dysfunctional or idiopathic pain. Inflammatory, neuropathic and dysfunctional pain can all feature reduced pain thresholds (hyperesthesia) and pain elicited by normally non-painful stimuli (allodynia), a phenomenon known as sensitization [98]. Pain sensitization can occur both in the peripheral and in the central nervous system as described below [99].

Notably, the sensation and the affective quality of pain is very subjective. It is influenced by many factors including, apart from differences in endogenous pain modulation and sensitivity, factors such as previous experience, preconceptions, personality and, especially in the case of dyspareunia, sexuality [19, 35, 46, 100].

5.4.1 Nociceptors and nerve fibers

Painful mechanical, thermal, and chemical stimuli are registered by peripheral nociceptors. These are morphologically free nerve endings without a specialized receptor structure, which express receptors for chemicals generated in tissue injury and immune response. Nociceptors can be classified according to several factors, including neurochemical profile, peptidergic/non-peptidergic type, and functional properties [101]. The pain signal is transmitted to the central nervous system via two types of axon: thin myelinated Aδ fibers and unmyelinated C fibers. Aδ fibers rapidly transmit discriminative information which leads to the first sharp, localized sensation of pain; the slower conduction in C fibers results in secondary aching or burning pain [102]. The cell bodies of these afferent fibers are in the dorsal root ganglia of the spinal cord. The fibers also contain neuropetides, which are transported out into the periphery and released upon activation of the nerve, resulting in efferent effects.

5.4.1.1 *Peripheral pain modulation*

Peripheral nociceptors have a dynamic phenotype and can be sensitized to give increased excitability and enhanced responsiveness by noxious stimulation or endogenous substances such as prostaglandins, leukotrienes and serotonin released during inflammation. There are also so-called sleeping nociceptors, which only become responsive during pathological conditions such as inflammation. Signals can also be increased by the influence of neuroactive substances such as CGRP and substance P released from neighboring neurons, causing neurogenic inflammation [103].

5.4.2 Spinal cord transmission

The terminal points of the primary afferent neuron are mainly in the laminae I, II and V of the spinal dorsal horn. Two major neurons receive the nociceptive input from the periphery: so-called nociceptive specific (NS) and wide dynamic range (WDR) neurons; these convey precise localized and more diffuse/larger-area information, respectively.

5.4.2.1 *Spinal pain modulation*

Intensive inter-neuronal networks at this level modulate the information before it is transmitted to the brain, and also connect to the efferent nerves of skeletal muscles and sympathetic fibers. In 1965 it was proposed that the incoming signals in the Aβ fibers of peripheral nerves, which transmit sensations of touch and vibration, could reduce the sensitivity of the post-synaptic cells to painful stimuli arriving in C and Aδ fibers, a phenomenon called the gate-control theory [104]. This finding led to many following studies exploring spinal pain modulation and we now know that it is a very complex phenomenon [105]. The Aδ and C fiber terminals and the dorsal horn inter-neurons contain both excitatory and inhibitory amino acids, as well as various neuropeptides, serotonin and endorphin. The amounts and regulatory effects of these substances are hugely variable in relation to different patho-physiological conditions [106, 107]. Repeated

painful stimuli can cause sensitization by increasing the excitability of spinal cord neurones in a frequency-dependent manner, called the wind-up phenomenon or temporal summation [108]. Furthermore, preclinical studies have demonstrated that microglia and astrocytes in the spinal cord are activated in experimental pain models, but the role of these cells in human pain modulation is still unclear [109, 110].

5.4.3 **Supraspinal and cortical centers**

Painful stimuli are further transmitted via the ascending spinothalamic tract which projects to the thalamus, and from there to the frontal or somato-sensory cortex. The anterior singulate cortex integrates information about pain perception. The autonomic response to pain is to some extent transmitted via the reticular formation in the brain stem which receives information via the spinoreticular tract.

5.4.3.1 *Central pain modulation*

Descending pain modulation can be either facilitatory or inhibitory. Two major receptors are involved: the inhibitory GABA receptor and the excitatory NMDA receptor [111, 112]. The role of central pain modulation in chronic pain states has been investigated to some extent; for example, MRI findings suggest impaired central pain inhibition in fibromyalgia patients [113]. One supra-spinal pain-modulating mechanism is constituted by pain from a primary stimulus being reduced by application of a second painful stimulus distant from the first; i.e. pain inhibits pain [114]. This phenomenon was first discovered in rodents and was initially known as diffuse noxious inhibitory control (DNIC). Today the phenomenon is called conditioned pain modulation (CPM) in human studies [115]. The inhibitory pathways descend from the caudal brain stem to the lamina II in the spinal dorsal horn. The inhibitory effect is executed at the spinal level mainly by serotonin and noradrenalin, resulting in inhibition of the release of substance P [116]. Deficiencies in CPM/DNIC function have been found in many chronic pain conditions, including TMD, tension headache, and fibromyalgia [117, 118]. However, PVD patients appear to have an intact DNIC response [38].

Moreover, the affective and aversive component of pain is modified at the cortical level [119, 120]. A clinical implication of cerebral pain modulation is the effectiveness of CBT in treating chronic pain [121]. A basic summary of pain transmission and modulation in the nervous system is shown in Figure 3.

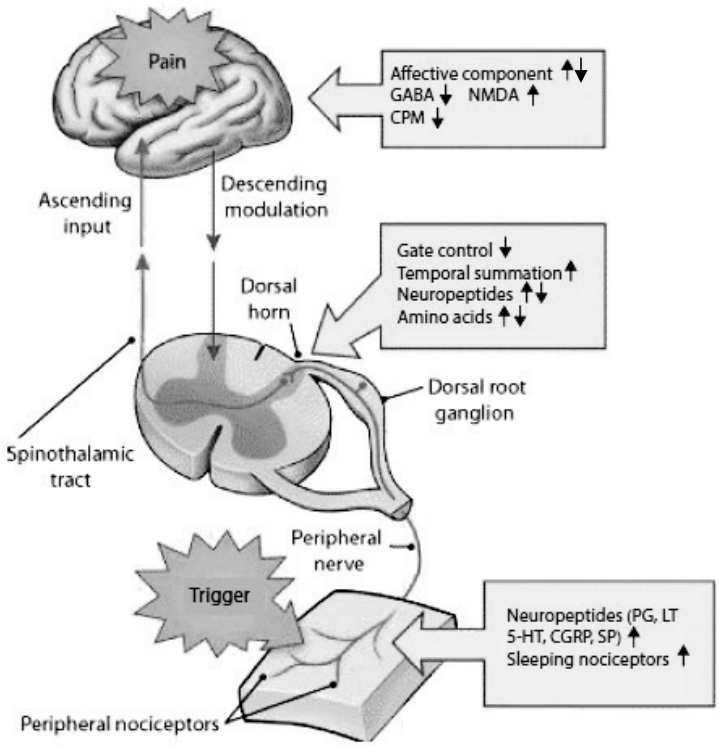


Figure 3. A basic summary of endogenous pain transmission and modulation at different levels of the nervous system.↑excitatory factors↓inhibitory factors

5.4.4 Pain modulation by sex hormones

Many pain conditions, such as TMD, tension headache, and fibromyalgia, are more prevalent in women than in men; in fact, more than 50% of the 77 most common pain disorders are more prevalent in women, whereas 30% of them appear not to be associated with sex [122]. This is thought to be related to the effects of the sex hormones [123]. Several studies have investigated the changes in pain sensitivity that occurs during the menstrual cycle [124-126]. Nociception-responsive neurons in the medullary dorsal horn of rats express ERα, which provides a possible morphological basis for the hypothesis that estrogens directly regulate pain transmission at this level [127, 128]. Pain sensitivity has been reported to be greater in the follicular phase than in the luteal phase in women with normal menstruation, although there are some inconsistent results [129, 130]. Kowalczyk et al. found no effect of the menstrual cycle on the pain threshold or tolerance to cold pressor pain, nor any difference in pain thresholds between COC users and non-users [131]. Exogenous reproductive hormones are associated with increased risk of TMD and may exacerbate migraine headaches. Fillingim et al. found lower pain tolerance in postmenopausal women using estrogen therapy; however, this finding was not

reproduced among female fibromyalgia patients [132, 133]. In a study by Johannesson et al., there were no differences in pressure pain thresholds (PPTs) on the arm or in the DNIC response between COC users and non-users examined during the follicular phase [38]. In contrast, Rezaii et al. found lower DNIC responses in healthy women using COCs than in non-users in the low estrogen phase, indicating less effective endogenous pain modulation in COC users, but with only a weak correlation to endogenous estrogen levels [134].

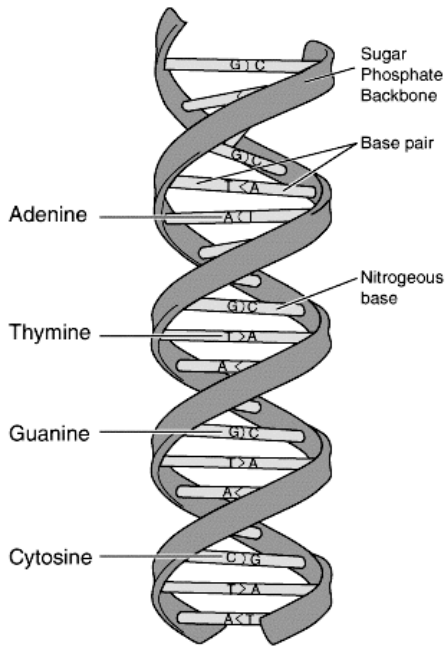
5.4.5 Endogenous opioids

β-endorphin, the endogenous agonist of the μ-opioid receptor, shares a common precursor, pro-opiomelanocortin (POMC), with adrenocorticotrophic hormone (ACTH), which is synthesized in the anterior pituitary gland and secreted into the peripheral blood in response to pain and other stressful stimuli. ß-endorphin is also released when descending pain inhibitory systems are stimulated. There is little information about the relationship between resting plasma levels of β-endorphin, endogenous pain modulation, and the functioning of the opioid system. Analgesic pathways for plasma β-endorphin are less clear than the central effects of β-endorphin in the cerebrospinal fluid (CSF). ß-endorphin levels in plasma and CSF do not necessarily correspond [135]. Elevated plasma β-endorphin levels have been suggested as a biomarker for reduced endogenous opioid antinociceptive function in chronic pain patients [136].

5.5 PAIN GENETICS

5.5.1 Introduction

The number of studies investigating the influence of genetic polymorphism on endogenous pain modulation is currently increasing [137]. There are a few monogenic disorders of pain, including the hereditary sensory and autonomic neuropathies that involve an absence of pain sensibility. However, in many chronic pain conditions without any structural lesions, the contribution of a single genetic polymorphism can be expected to be only modest, and a wide variety of genes have been associated with both clinical and experimental pain. The wide variability in the development of chronic pain syndromes per se and the inter-individual variability in the intensity of pain are a great challenge to genetic pain research. It is thought that a triggering insult such as an infection or trauma is required for a chronic pain condition to develop, but so too are susceptibility factors that might be inherited. This gene-environment interaction could lower the sensitivity of genetic studies. Twin studies of chronic pain syndromes have shown estimates of heritability ranging from 13% to 50% [138-141]. Many genetic pain studies focus on Mendelian or dominant models, i.e. one copy of the minor allele confers the maximal difference in phenotype from the homozygous for the major allele. Interest in the role of gene-environment, gene-sex, and gene-gene interactions has increased in recent years. For example, desmopressin analgesia was shown to result from a three-way interaction between arginine vasopressor receptor gene variant (AVPR1A), sex, and level of stress [142].



There are contradictory data regarding whether there are genetic factors common to multiple pain syndromes or symptoms or whether completely different genes underlie different pain disorders and possibly even different pain modalities such as thermal, mechanical or ischemic pain. Furthermore, there is uncertainty whether an association between a genetic polymorphism and a pain syndrome is related to pain processing per se or to the psychological modulators of pain, or to both [137].

One of the most common types of gene polymorphism is a single nucleotide polymorphism (SNP), where one nucleotide in the DNA molecule is replaced by another; if this polymorphism is located in the exon, it could alter the properties of the corresponding protein/peptide.

Figure 4. The DNA helix with nucleotide base couples.

5.5.2 Genetic studies

There are several ways to carry out a genetic study. In genome-wide screening (GWS), multiple markers are used to search every human gene for susceptibility loci. Recent technical advances have meant that genotyping is quicker and less expensive, making GWS more feasible; however, these studies often require co-operation between many centers, with sample sizes into the thousands, to overcome statistical problems with the multiple testing. GWS has historically been more widely used in other fields of biomedicine, but is now increasingly used also in the field of pain. In another approach, family linkage studies use several hundred genetic markers to search the entire genome of related subjects, who share whole chromosomes, for susceptibility loci. A third alternative is candidate gene association studies. This approach has been widely used in the field of pain research, with an acceleration of findings in recent years. In association studies, the frequencies of common allelic variants in specified genes are compared between patients and controls. In the pain field association studies have so far focused on a limited set of candidate genes; 10 genes, including the guanosine triphosphate cyclohydrolase 1 (GCH1), μ-opioid receptor 1 (OPRM1), and serotonin receptor 2A (5HT-2A) genes, account for over half of the findings to date [137]. However, replication of association studies has resulted in largely inconsistent or contradictory findings, possibly due to problems with sample size and study design, with differing inclusion/exclusion criteria, pain assessment methods, environmental testing conditions, etc. In fact, to date, no genetic association in the field has been consistently replicated and none has explained a large proportion of trait variance, a fact that supports the value of GWS studies and more complex approaches in this field in the future.

Furthermore, recent work suggests that micro-RNA and epigenetic mechanisms are involved in the regulation of gene expression and pain modulation [143, 144], which increases the complexity of the picture even more.

5.5.3 **GCH1**

In 2006 Tegeder and colleagues reported that specific SNPs in the *GCH1* gene are associated with reduced pain sensitivity in humans [145]. The *GCH1* gene is coding for GTP cyclohydrolase; the rate-limiting enzyme in the biosynthesis of 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4). BH4 is an essential cofactor in the synthesis of several pain modulators including catecholamines, serotonin and nitric oxide. BH4 regulates the activity of *GCH1* via feed-forward activation of phenylalanine and feedback inhibition. The identified pain-protective haplotype of *GCH1* is composed of 15 SNPs found at different locations on the gene. Screening for three of these SNPs has been shown to be a reliable way to identify the pain-protective haplotype with high sensitivity and specificity [146]. At the biochemical level the haplotype has been demonstrated to result in decreased GTPcyklohydrolase upregulation and BH4 production following stimulation; see Figure 5 [147].

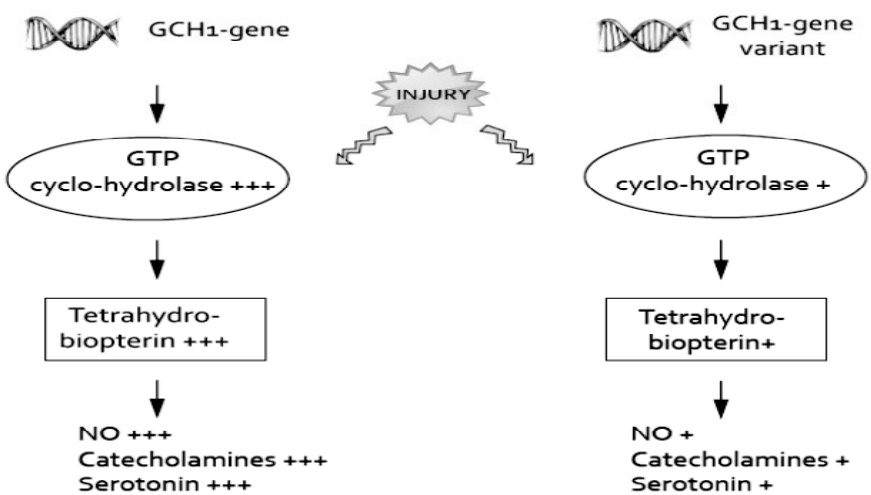


Figure 5. A schematic presentation of the 15 SNP haplotype of the *GCH1* gene resulting in reduced production of pain excitatory substances after stimulation.

Several studies, but not all, have linked *GCH1* polymorphism with various aspects of pain, including neuropathic and inflammatory pain [148-150]. The studied *GCH1*-SNP combination has been associated with protection from the development of chronic pain after surgery for lumbar disc hernia and degeneration [145, 151] but not of chronic pain after surgical removal of molar teeth [152] or of chronic wide-spread pain [153]. The most robust associations between *GCH1* and pain responses have appeared in acute inflammatory pain models. Protective effects

of the SNP combination against mechanical and thermal pain have been found when measuring experimental PPTs after induced hyperalgesia of the skin through freezing or applying capsaicin [147, 154]. A study investigating a possible association between different SNP combinations in the *GCH1* gene and a number of pain behavior-related outcomes during labor indicated a very limited effect [155].

5.5.4 **OPRM1**

The importance of the opioid system in both endogenous and exogenous pain modulation is well known. Substantial attention has been focused on the impact of polymorphisms in the *OPRM1* gene. The SNP A118G (rs1799971) in the *OPRM1* gene causes a substitution from asparagine to aspartic acid at amino acid 40, with the resultant removal of a putative N-linked glycosylation site in the receptor and effects on endogenous pain modulation [156]. Increased β-endorphin potency and increased receptor-binding affinity between β-endorphin and the variant 118G receptor have been proposed, see Figure 6 [156]. However, results are equivocal; in 2004, Beyer et al. reported similar β-endorphin binding affinities and potencies for both receptor variants (118G and 118A) [157].

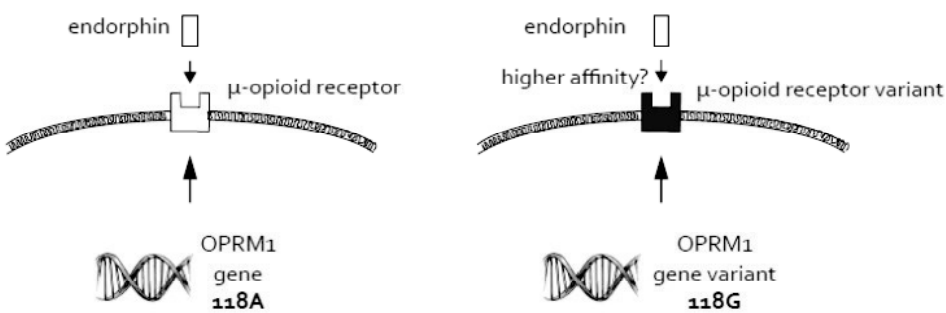


Figure 6. A schematic presentation of the A118G SNP in the *OPRM1* gene resulting in an altered μ-opioid receptor with a supposedly higher binding affinity of β-endorphin.

In healthy individuals, the 118G allele was initially thought to be pain-protective, with reports of carriers having higher PPTs and less chronic pain than non-carriers [158, 159]. Fillingim et al. reported that healthy individuals with heterozygous (AG) and minor homozygous (GG) genotypes had higher PPTs than individuals with the major homozygous (AA) genotype [157]. However, recent studies show a more complex picture, with somewhat conflicting results; the association between A118G polymorphism and pain sensitivity seems to be influenced by factors such as sex, ethnicity, and pain modality [160, 161]. For example, women homo- or heterozygous for the 118G allele experienced higher pain intensity in the first year after lumbar disc herniation and reported more pain following cesarean section than 118A carriers [162, 163].

5.5.5 **5HT-2A**

Serotonin is the key neurotransmitter in the serotonergic system. This system has wide-ranging actions throughout the body, including an antinociceptive role in the dorsal horn of the descending tract of the spinal cord [164-166]. Selective serotonin reuptake inhibitors (SSRI) have been shown to be effective in the treatment of depression, anxiety and pain conditions such as fibromyalgia and CWP [167]. However, it is unclear whether the effect of SSRI-treatment in fibromyalgia is due to effects on pain processing or on the common co-morbid symptoms of depression. The serotonin receptor gene, *5HT-2A*, has been well researched; studies have reported two common SNPs in this gene: A-1438G and T102C. These SNPs appear always to be co-inherited, a so-called complete linkage disequilibrium [168]. The A-1438G/T102C polymorphism does not alter the amino acid composition, and therefore has no influence on the receptor protein; therefore, linkage disequilibrium to the causative mutation has been proposed as a mechanism for the reported associations [169].

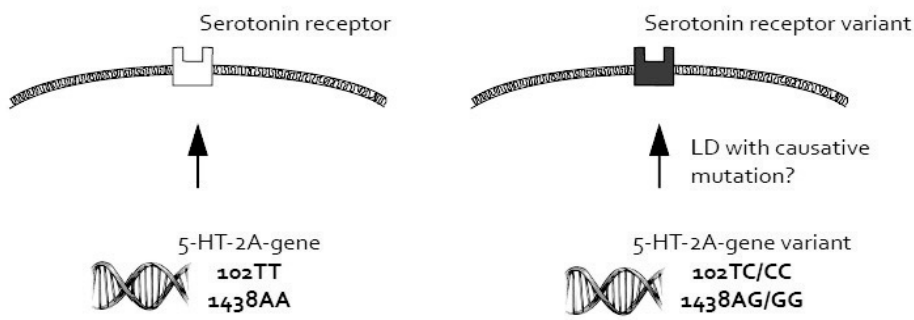


Figure 7. A schematic presentation of the A-1438G and T102C SNPs in the 5HT-2A gene.

A review by Lee in 2012 concludes that there is a significant association between the CC+CT genotype of the T102C SNP and fibromyalgia [170]. Similarly, the T allele of the T102C SNP has been associated with a decrease in the number of somatic symptoms in a British population survey [171]. In contrast, in a group of fibromyalgia patients, carriers of the TT genotype reported higher pain scores [169]. There are also reports of an association between the A-1438G/ T102C SNPs and depression [172, 173].

6 AIMS

The main aim of these studies was to investigate a possible genetic predisposition for the development of PVD, with particular focus on three genes known to influence endogenous pain modulation: the *GCH1*, *OPRM1* and *5HT-2A* genes.

Other aims included investigation of:

I. possible predictors of treatment outcomes and prevalence of pain co-morbidity in women with PVD (Study I);

II. a possible interaction between polymorphisms in the *GCH1* gene and use of oral contraceptives with effects on pain sensitivity in women with PVD and healthy controls (Study II);

III. a possible correlation between polymorphisms in the *OPRM1* gene and serum levels of β-endorphin, and effects on pain sensitivity in women with PVD and healthy controls (Study III); and

IV. a possible correlation between polymorphisms in the *5HT-2A* gene and symptoms of anxiety or depression, and effects on the risk of developing PVD and pain sensitivity in women with PVD and healthy controls (Study IV).

7 PARTICIPANTS

7.1 ETHICS

The studies were approved by the local ethics committee at Karolinska Institutet and all participants received oral and written information about the studies and provided written, informed consent.

7.2 SUBJECTS

The studies were carried out between May 2008 and May 2010. The four studies involved a total of 109 women with PVD and 103 healthy controls.

7.2.1 Women with PVD

Most of the participants were former or current patients at the vulvar open care unit at Danderyds Hospital. In addition, a smaller group (n = 6) was recruited from three other gynecological open care units in the same area. Ninety-eight of the PVD patients completed the whole study and 11 participated by answering questionnaires only. The inclusion criteria for patients were: age ≥ 18 years, PVD defined as pain on vestibular contact and vaginal entry, no current local infection or dermatological causes of dyspareunia, and a minimum 6 months' duration of symptoms based on the initial examination at the time of diagnosis. The exclusion criteria were: major psychiatric or medical disease and pregnancy.

7.2.1.1 Recruitment

Inquiries inviting women to participate were sent by mail to patients who had received treatment for PVD between 1997 and 2008 or who were currently receiving treatment, according to their medical records. One hundred and ninety-three women were contacted by mail at the start of the project, and an additional letter re-enquiring about their willingness to participate was sent a year later to those who had not responded. Sixty-seven women agreed to join the study. Patients currently receiving treatment where contacted by a research nurse. Forty-three additional patients were enrolled during the test period; of these, seven had completed treatment and 36 were still receiving treatment.

7.2.1.2 Participants in the four studies

Study I enrolled only patients who had completed treatment for PVD, including those who only answered questionnaires (n = 70).

Studies II-IV enrolled all patients who fulfilled the inclusion/exclusion criteria and underwent the complete testing (n = 98). One participant with generalized vulvodynia was excluded for not fulfilling the diagnosis criteria.

7.2.2 Controls

One hundred and two healthy controls were recruited via advertisement at medical schools and hospitals in the Stockholm area for Studies II-IV; respondents were mostly medical students and

hospital staff. The inclusion criteria were age > 18 years, and regular menstruation. Exclusion criteria were: dyspareunia, major medical or psychiatric disease, use of regular painkilling or antidepressant medication, and pregnancy.

8 METHODS

8.1 QUESTIONNAIRES

Participants were invited to a single testing session, carried out in the follicular phase (days 3-13) of the menstrual cycle, in order to standardize any differences in mood or pain perception during the menstrual cycle [174, 175].

8.1.1 Study-specific questionnaires

All participants filled out a study-specific questionnaire surveying age, occupation and medical (including gynecological and psychosocial) history, as well as bodily pain symptoms, including dysmenorrhea. The pain symptoms were divided into five categories: headaches, muscle pain, gastrointestinal pain, back pain and any other pain. The number of bodily pain disorders was used as an index to create an overall bodily pain score, ranging from 0 to 5. Dysmenorrhea was not included in the pain score. Present or previous use of hormonal contraceptives (HCs) was reported.

The patients in Studies I-IV also completed a second questionnaire containing questions related to PVD such as the duration of symptoms, whether the symptoms had a primary or secondary onset, and what treatments had been used. The intensity of coital pain during the last month was scored on a visual analog scale (VAS) ranging from 0 to 100, where 0 represented no pain and 100 represented the worst pain imaginable. Participants were also asked to define coital pain during the last month by choosing one of the following options: (a) never pain, (b) occasional mild pain not preventing vaginal intercourse, (c) moderate pain sometimes preventing vaginal intercourse, or (d) severe pain making vaginal intercourse impossible. Patients who had completed treatment rated their treatment outcome by choosing one of the following options: (a) no change, (b) improvement, (c) major improvement, or (d) complete recovery. (See Appendix on page 57 for an English translation of the questionnaires.)

8.1.2 HADS

A psychometric screening questionnaire, the Hospital Anxiety and Depression Scale (HADS), was filled out by all participants of Studies I and IV [176, 177] to detect anxiety and depression disorders. HADS is a validated screening instrument that has been found to perform well in assessing the symptom severity and caseness of anxiety disorders and depression in both somatic and primary care patients as well as in the general population. It is composed of seven statements related to anxiety and seven related to depression. Each statement is ranked from 0 to 3, with 0 representing no symptoms and 3 representing considerable symptoms. The maximum score for each symptom is 21, with a score ≥ 8 indicating mood affection and a score ≥ 11 suggesting the presence of a mood disorder [178].

8.2 QUANTITATIVE SENSORY TESTING

8.2.1 Peripheral pressure pain thresholds

PPTs on the arm and leg were measured for all participants (Studies II-IV) using a pressure algometer (Somedic Sales AB, Hörby, Sweden) with a disc-shaped rubber top 1 cm^2 in diameter; see Figure 8.

Figur 8. Pressure algometer

The arm was tested first, on the deltoid muscle 3 cm proximal to the tendon insertion. The leg was tested on the anterior tibial muscle approximately 5 cm below and 3 cm lateral to the tibial tuberosity. Testing was performed on the side opposite to the reported dominant hand. The device was applied perpendicularly to the skin and the pressure was increased by 50-75 kPa/s. The participants were asked to report the PPT, which was defined as the point at which the sensation changed from discomfort to the first sensation of pain, by pushing a button. The pressure at this point, displayed digitally, was then registered. The measurement was repeated twice and the mean value was registered. All participants were given a careful explanation of the procedure and a training session on the opposite arm before the testing started. Measurements were carried out by one examiner who was blinded to whether the participant was a patient or a control.

8.2.2 Vestibular pressure pain thresholds

Patient or control status was revealed for all participants (Studies II-IV) after testing PPTs on the arm and leg. PPTs in the vestibular mucosa were then measured in patients only, using vulvar algesiometers [179]. The algesiometers consisted of cylindrical devices containing metal springs of varying compression rates with a cotton swab at one end. The set was calibrated to exert pressures ranging from 3 to 1000 g.; see Figure 9a. Two areas of the vestibule were tested: area A was in the anterior vestibule, close to the urethra, and area B was in the posterior vestibule, close to the opening of the Bartholin´s glands; both were on the right side of the vaginal opening, as shown in Figure 9b. The pressure was successively increased until the participator orally reported the PPT, as described above. The measurement was repeated twice and the mean value was used for analysis. All subjects were given a careful explanation of the procedure before the testing started.

a) **b)**

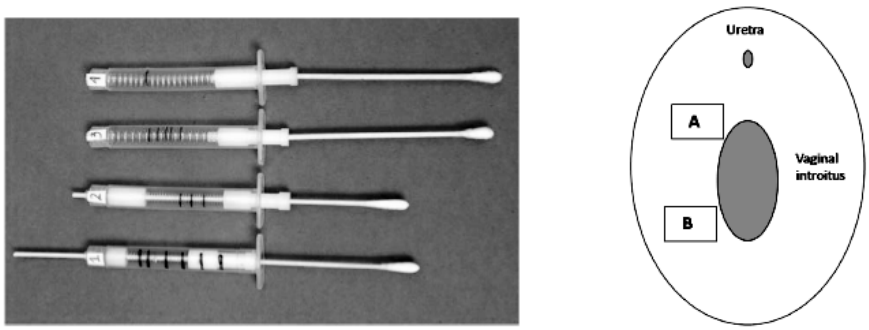


Figure 9a) Vulvar algesiometers. 9b) Areas A and B indicating where pressure pain thresholds were measured in the vestibulum.

8.3 ANALYSES OF GENES AND ENDORPHIN LEVELS

8.3.1 Sample collection

Venous blood samples were collected in tubes containing ethylene diamine tetra-acetic acid (EDTA). Whole blood samples were centrifuged for 10 minutes at 3000 rpm, and plasma was collected for radioimmunoassay (RIA) analysis of ß-endorphin levels. The blood samples were stored at -70° C until further processing.

8.3.2 DNA isolation

The genetic analyses were performed at the Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, and the Genome Center, Uppsala University, Uppsala, Sweden. The Magtration 12GC system (Precision System Science, Chiba, Japan) and the Magazorb® DNA Common Kit-200 (PSS, Chiba, Japan) were used for preparation of the total genomic DNA. From each sample, 200 µl of whole blood was taken to provide a final volume of DNA extract of 100 µl. The concentration of the DNA was determined with a Nanodrop Spectrophotometer (Nanodrop Techncologies Inc., Wilmington, DE, USA).

8.3.3 Genotyping

- In Study II, three SNPs were analyzed to define the pain-protective haplotype of *GCH1*: rs8007267 (c.-9610G > A), rs3783641 (c343+8900A > T) and rs10483639 (c.*4279 > G).
- In Study III, the rs1799971 (A118G) SNP was analyzed in the *OPRM1* gene.
- In Study IV, two SNPs, rs6313 (T102C) and rs6311 (A-1438G), were analyzed in the *5HT-2A* gene (assay numbers C_3042197_1 and C_8695278_10, respectively).

The TaqMan SNP genotyping assay (Applied Biosystems, Foster City, USA) was used to analyze these SNPs. Briefly, Applied Biosystems designed the primers and the allele-specific probes. The assay included target-specific PCR primers and TaqMan MGB probes labeled with two special dyes, FAM and VIC. Genomic DNA (5 ng), water, TaqMan Universal PCR master mix, and TaqMan genotyping assay mix were added to each well in a 384-well plate, to a total volume of 5ul. The genotyping was carried out using the ABI7900HT genetic detection system (Applied Biosystems, Foster City, USA) according to the manufacturers´ instructions, with the following amplification protocol: 10 min at 95°C, 40 cycles of 15 s at 92°C, and 1 min at 60°C.

8.3.4 **Radioimmunoassay of β-endorphin**

The frozen plasma samples taken in Study III were thawed on ice and centrifuged at 4°C for 10 min at 3000 x g. The supernatants were collected, diluted (1:5) with 0.1 M formic acid and 0.018 M pyridine (buffer I), and separated on minicolumns (1 ml) packed with SP-Sephadex C-25 gel. The columns were washed with 10 ml buffer I prior to sample application, and 10 ml buffer I and 5 ml 0.1 M formic acid/0.1 M pyridine (pH 4.1; buffer II) after sample application. The peptide-containing fractions were then eluted with 4 ml 1.6 M formic acid/1.6 M pyridine (pH 4.1; buffer V). All buffers contained 0.01 % mercaptoethanol. The eluted samples were evaporated in a Speed Vac centrifuge (Savant, Hicksville, NY, USA).

The EURIA-beta-endorphin kit (EURO-DIAGNOSTICA AB, Sweden) was used for the ß-endorphin RIA, which was based on double-antibody precipitation. The evaporated samples were diluted with 220 µl diluent (0.05 M phosphate pH 7.4, 0.25% human serum albumin, 0.05% sodium azide, 0.25% EDTA and 500 KIU Trasylol®/ml) and incubated with 100 µl of anti-ß-endorphin antiserum for 24 h at 4° C. After incubation, the labeled peptide, ^{125}I-ß-endorphin, was added to each sample and incubated for an additional 24h at 4°C. Thereafter, the double antibody PEG was added, and the tubes were incubated for 60 min and then centrifuged for 15 min at 12000 rpm and 4°C. The supernatants were then decanted and the radioactivity of the precipitates was counted in a gamma counter.

8.4 **STATISTICS**

The Statistica program (version 10, StatSoft Inc., Tulsa, OK, USA) and the Statistical package for the Social Sciences program (version 20, SPSS Inc., Chicago, IL, USA) were used to analyze the data. The student's t-test and the Mann-Whitney U-test were used to analyze continuous numeric data and ordinal and non-normally distributed data, respectively, for comparisons between groups regarding age, clinical background data, pain measurements, HADS scores and β-endorphin levels. The Chi-square test and Fishers exact test were used to analyze frequencies for comparisons between groups regarding clinical background data, HADS scores dichotomized (<8/≥8) and SNPs. A significance level of $p < 0.05$ was used for all statistical tests and a confidence interval of 95% was used for the logistic regression analyses.

Study-specific analyses were:

- Study I: a multivariate logistic regression analysis and the Mann-Whitney U-test were used to identify variables associated with treatment outcome. A multivariate linear regression analysis was carried out in order to explore possible correlations between the coital VAS pain score (dependent variable) and the other variables (PPTs on the arm and leg, bodily pain scores, anxiety, depression, previous depression treatment, duration of PVD, primary or secondary PVD, duration of use of HCs, and the degree of coital pain).
- Study II: pain sensitivity between groups was compared using the analysis of variance (ANOVA) test. The nonparametric equivalents Kruskal-Wallis and the Mann-Whitney U-test were used for ordinal data. General linear regression models (GLM) were used to detect a possible interaction between the *GCH1*-SNP combination and use of HCs with respect to pain sensitivity.
- Study III: logistic regression was used to explore a possible association between the A118G polymorphism and a diagnosis of PVD, and the Spearman rank method was used to investigate possible correlations between β-endorphin levels and pain measurements.
- Study IV: univariate and multivariate logistic regression methods were used to explore associations and interactions between the *5HT-2A* polymorphism, HADS scores, pain measurements, and PVD.
- Unpublished data: three methods were used to measure the degree of vestibular and coital pain: PPTs in the vestibulum and coital pain levels self-rated on a VAS and by choosing one of the following options: (a) never pain, (b) occasional mild pain not preventing vaginal intercourse, (c) moderate pain sometimes preventing vaginal intercourse, or (d) severe pain making vaginal intercourse impossible (see methods). A Cronbachs-alpha analysis was performed to investigate the inter-item correlation matrix.

9 RESULTS

9.1 CLINICAL BACKGROUND DATA

Clinical background data for the patients and controls in Studies I-IV are shown in table 2.

Table 2. Clinical data on participants.

Variables	Patients (n=109)	Controls (n=102)	p-value
Demographic data			
Age, years (range)	29.2 (19-44)	24 (18-35)	<0.001
Currently studying	16 (15%)	67 (66%)	<0.001
Currently employed	65 (61%)	17 (17%)	<0.001
Studying + employed	20 (19%)	17 (17%)	ns
Current with a partner	81 (76%)	-	
Caucasian ethnicity	96%	97%	ns
Reproductive data			
Ever use of HCs	73 (68%)	60 (59%)	ns
Use of HC, years (range)	7.2 (0.1-22)	4.2 (0.1-18)	<0.001
Currently using HCs	35 (33 %)	55 (54%)	0.005
Combined HCs	26 (27%)	42 (40%)	ns
Progestogen only HCs	7 (7%)	11 (11%)	ns
Regular menstruation	83 (80 %)	94 (93%)	0.014
Menstrual cycle day	8 (4-13)	8 (3-13)	ns
Given birth	18 (17 %)	7 (7%)	0.028
Vaginal delivery	17 (16 %)	6 (6%)	0.022
Medical history			
Eczema	34 (33%)	16 (16%)	0.004
Asthma	19 (19%)	11 (11%)	ns
Allergy	36 (35%)	15 (15%)	<0.001
Previous treatment for depression	70 (65%)	29 (28%)	<0.001
Current SSRI-treatment	6 (6%)	0 (0%)	0.01
PVD			
Primary PVD	39 (36%)	-	
Secondary PVD	70 (64%)	-	
Duration of symptoms, years	10.8 (0.5-23)	-	
Ongoing treatment	38 (37 %)	-	
Completed treatment	70 (63%)	-	

Patients with completed treatment were followed for a median of 5 years (range 2 months to 11 years). Eleven (65%) of the 17 women with PVD who had delivered vaginally reported unchanged pain intensity after birth, while the rest reported improved status.

9.2 PAIN CO-MORBIDITY

Pain co-morbidity was measured in Studies I-IV. Patients reported more frequent pain symptoms than controls in all the pain categories; dysmenorrhea was the most frequent complaint (see table 3). Women with secondary PVD reported dysmenorrhea significantly more often than those with primary PVD ($\chi 2 = 4.99$, $p = 0.03$). Twenty participants reported another pain in addition to the specified pain categories, most commonly joint pain (n = 6). One participant reported TMD and one reported fibromyalgia. There were no reports of bladder pain or urethritis. No participant reported more than one other additional pain. A bodily pain score ≥3 was obtained in approximately one third of the participants.

Table 3. Patients self reported frequent pain symptoms

Concomitant pain	Patients (n=109)	Controls (n=102)	p-value
Dysmenorrhea	74 (72%)	55 (54%)	0.02
Headache	65 (61%)	30 (29%)	<0.001
Muscle pain	32 (31%)	2 (2%)	<0.001
GI pain and dysfunction	57 (54%)	22 (22%)	<0.001
Back pain	50 (48%)	20 (20%)	<0.001
Any other pain	30 (47%)	1 (1%)	<0.001

9.3 HADS

Screening scores for both anxiety and depression were significantly higher in women with PVD than in healthy controls in Studies I and IV, (total scores in patients versus controls: HADS anxiety; p<0.001, z= -4.588, HADS depression: p=0.002, z=-3.035). Proportions of scores ≥8 are shown in Table 4. More than half of the patients reported heightened anxiety levels, and one-third of these had a HADS score indicating an anxiety disorder. Significantly more patients with secondary PVD reported a HADS anxiety score ≥8: 63% compared to 46% of patients with primary PVD ($p = 0.05$), but there were no differences between the groups regarding proportions of an anxiety score ≥11. No differences were found regarding levels of anxiety symptoms between women with PVD who were currently receiving treatment and those who had completed treatment. Heightened levels of depressive symptoms were found in 10% of the patients, of whom one had a result indicating a manifest depression.

Table 4. Proportions of HADS scores ≥8 in patients and controls

HADS score	Patients (n=109)	Controls (n=102)	p-value
Anxiety Score ≥8	56 (57%)	21 (20%)	<0.001, χ^2=28.706
Depression Score ≥ 8	10 (10%)	1 (1%)	0.004, χ^2=8.276

9.4 PAIN MEASUREMENTS

Pain sensitivity was significantly higher in patients than in controls in Studies II-IV, for all the measured pain modalities, with lower experimental PPTs on the arm and leg and higher self-reported bodily pain scores in patients, as shown in Table 5.

Table 5. Pain measurements in patients and controls

Pain variables	Patients (n= 98)		Controls (n=103)		p-value
	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	
PPT leg (kPa)	405 (161)	390 (299-499)	474 (152)	457 (361-575)	0.002 (t-test) 0.001 (M-WU)
PPT arm (kPa)	268 (124)	238 (189-331)	309 (116)	298 (227-355)	0.018 (t-test) 0.002 (M-WU)
Bodily pain score (0-5)	2.1 (1.2)	2 (1-3)	0.7 (0.9)	0 (0-1)	< 0.001 (M-WU)
PPT vest A (g)	48 (31)	40 (25-60)	-	-	-
PPT vest B (g)	42 (44)	28 (15-50)	-	-	-
Coital VAS pain (0-100)	53 (32)	54 (23-78)	-	-	-

In Study I, a multiple linear regression model showed an association between coital VAS pain score and the number of other pain disorders with higher VAS scores in women with more pain disorders ($p < 0.01$), and between the VAS score and a diagnosis of primary or secondary PVD with higher VAS scores in the primary PVD group ($p = 0.04$).

9.5 TREATMENT OUTCOMES

The outcomes of treatment for PVD as self-reported in Study I are displayed in Table 6. The outcome was significantly better in the secondary PVD group than in those with primary PVD ($z = 2.11$, $p = 0.04$). The patients had received a mean of 2,5 different treatment modalities. Of the patients who reported major improvement or complete recovery, 63% had received no more than two different treatment modalities.

Table 6. Patient self reported treatment outcome after individually tailored multi-modal treatment at the vulvar open care clinic at Danderyd Hospital.

Treatment outcome	All patients (n=70)	Primary PVD (n=23)	Secondary PVD (n=47)
No change	13 (19 %)	8 (35 %)	5 (11 %)
Improvement	25 (36 %)	8 (35 %)	17 (36 %)
Major improvement	26 (37 %)	5 (22 %)	21 (45 %)
Complete recovery	6 (9 %)	2 (9 %)	4 (9 %)

Current PVD status differed between women with PVD currently receiving treatment (n=70) and those who had completed treatment (n=38). Moderate to severe pain was reported by 95% in the current-treatment group compared to 49% in the completed-treatment group ($\chi 2 = 33.8$, $p < 0.001$). No differences were seen between women with primary and secondary PVD.

Patients who had completed treatment had higher PPTs in vestibular area B than those currently receiving treatment: mean 51 g versus 27 g ($t = 2.64$, $p < 0.01$; see Figure 10). No statistically significant differences were seen in area A. There were no differences in vestibular PPTs between patients with primary and those with secondary PVD.

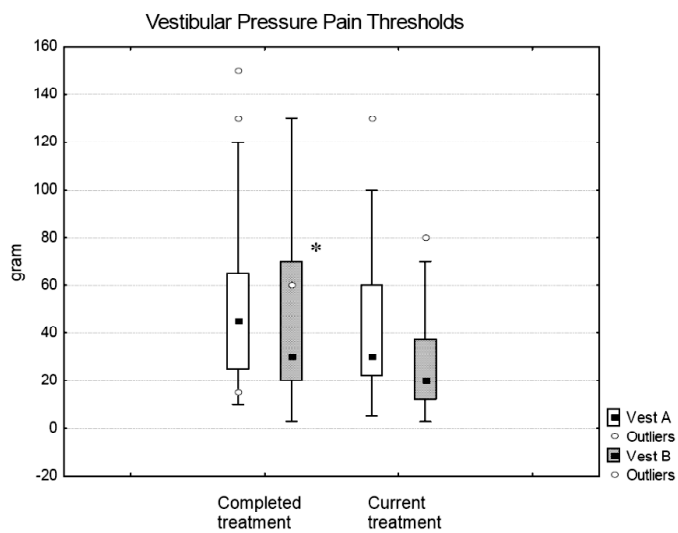


Figure 10. Lower vestibular pressure pain thresholds seen in area B (posterior vestibule) in women with PVD currently receiving treatment (n=38) than in women with completed treatment (n=70). * p<0.01. No difference seen in area A (anterior vestibule).

Coital VAS pain scores for participants who reported major improvement/complete recovery (median 22, range 0-58) were significantly lower than those for participants reporting no change/improvement (median 49, range 7-100; $z = 4.12$, $p < 0.001$). There were no differences in coital VAS pain scores in participants with completed treatment between those with primary PVD (median 33, range 0-91) and those with secondary PVD (median 24, range 0-100).

There was a strong correlation between self-rated coital pain defined by choosing one of four options as described above and coital VAS pain scores ($\alpha = 0.85$; see Figure 11), but only weak correlations between self-rated coital pain and PPTs in vestibulum areas A ($\alpha = 0.22$) and B ($\alpha = 0.18$).

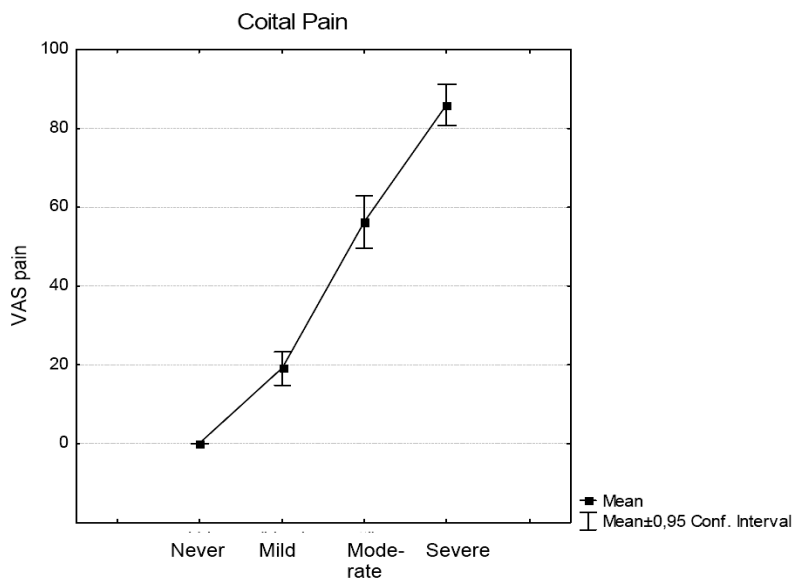


Figure 11. Correlation between patient self rated coital pain on a visual analog scale (VAS) and by choosing one of four expressions of pain intensity ($\alpha = 0.85$).

9.6 PREDICTORS OF TREATMENT OUTCOME

The number of other bodily pain symptoms was the strongest predictor of treatment outcome in Study I. Women with fewer other pain disorders were more likely to respond better to treatment, with an odds ratio (OR) of reaching an outcome of much better or complete recovery that was eight times higher among participants with no more than one other pain disorder compared to participants with four or more other pain disorders ($OR = 7.8$, CI: 1.2 - 49.4, $p = 0.029$). In a logistic regression model including the bodily pain score, the subgroup of PVD,

and PPTs on the arm and leg, only the bodily pain score was significantly associated with treatment outcome.

9.7 GENETIC FINDINGS

Genotyping for the studied SNPs was completed for 200 subjects: 98 patients and 102 controls. The frequencies of the SNPs were all in accordance with the Hardy-Weinberg equilibrium.

9.7.1 SNP frequencies

9.7.1.1 GCH1

The frequencies of the studied SNP combination in the *GCH1* gene (rs8007267G > A, rs3783641A > T, and rs10483639C > G) are shown in table 7. Individuals were classified as homozygous for, heterozygous for or non-carriers of the SNP combination, according to a previously outlined method [146].

Table 7. Frequencies of studied SNP-combination in the *GCH1* gene

GCH1 – gene	**Non-carriers** n (%)	**Homozygous carriers** n (%)	**Heterozygous carriers** n (%)
All participants (n=201)	139 (70)	5 (2)	56 (28)
Patients (n=98)	70 (71)	3 (3)	25 (26)
-ongoing treatment (n=38)	28 (73)	1 (3)	9 (24)
-completed treatment (n=60)	42 (70)	2 (3)	16 (27)
Controls (n=102)	69 (68)	2 (2)	31 (30)

Because of the low number of individuals who were homozygous for the SNP combination, homozygous and heterozygous individuals were merged into one group and compared with non-carriers in all further analyses.

9.7.1.2 OPRM1

The frequencies of the studied SNP (rs1799971) in the *OPRM1* gene are shown in table 8. Subjects who had minor homozygous (GG) or heterozygous (AG) status were combined to form the rare allele genotype (118G) group for further analysis, in accordance with previous studies [158, 160].

Table 8. Frequencies of the A118G SNPs in the *OPRM1* gene

OPRM1 -gene	118A	118G		p-value
	AA n (%)	**AG** n (%)	**GG** n (%)	
All participants (n=201)	127 (63)	58 (29)	16 (8)	
Patients (n=98)	69 (70)	24 (25)	5 (5)	0.042
Controls (n=103)	58 (56)	34 (33)	11 (11)	χ^2=4.29

9.7.1.3 5HT-2A

The frequencies of the studied SNPs (rs6313 and rs6311) in the *5HT-2A* gene are shown in Table 9. There was complete linkage disequilibrium between the two SNPs.

Table 9 Frequencies of the studied SNPs in the *5HT-2A* gene

5HT-2A - gene	T102C (rs6313)			p-value
	TT n (%)	**TC** n (%)	**CC** n (%)	
Patients (n=97)*	8 (8.2)	53 (54.6)	36 (37.1)	0.05
Controls (n= 103)	21 (20.4)	49 (47.6)	33 (32.0)	χ^2 = 5.94
5HT-2A - gene	**A-1438G (rs6311)**			**p-value**
	AA n (%)	**AG** n (%)	**GG** n (%)	
Patients (n=98)	8 (8.2)	53 (54)	37 (37.8)	0.05
Controls (n=103)	21 (20.4)	49 (47.6)	33 (32.0)	χ^2 = 6.09

*One patient missing due to error in analysis.

9.7.2 *GCH1* polymorphism and PVD

There were no differences in SNP frequency between patients (with current and/or completed treatment) and controls. Nor were there any differences in SNP carrier frequency between patients with primary or secondary PVD.

9.7.3 *GCH1* polymorphism, HC use, and pain sensitivity

There were no differences in PPTs, bodily pain scores or coital VAS pain scores between carriers and non-carriers of the defined SNP combination in patients or controls analyzed separately, or in the total sample analyzed together.

There were no significant differences between carriers and non-carriers in PPTs on the arm or leg for patients, controls or all participants together, irrespective of HC use. Among patients,

there were no differences between carriers and non-carriers in vestibular PPTs, coital VAS pain scores, or bodily pain scores, irrespective of HC use in general or use of combined or progestogen-only HCs. When all patients were analyzed together, there was a trend for an interaction of the specified SNP combination for *GCH1* and use of HCs with effect on the coital VAS pain score ($p < 0.07$), but with a low explained variance.

However, when women with PVD who were currently receiving treatment were analyzed separately, the *GCH1* gene variants had significant effects, and the interaction effect with HC use was also significant. The combined effect of the *GCH1*-SNP combination and HC use explained approximately 8% of the variance in the reported coital VAS pain scores. Among women with PVD receiving current treatment who were not using HCs (n = 23), carriers of the specified *GCH1*-SNP combination reported lower coital VAS pain scores than non-carriers. On the other hand, in the group using HCs (n = 15), carriers of the SNP combination reported higher coital VAS pain scores, as shown in Figure 12.

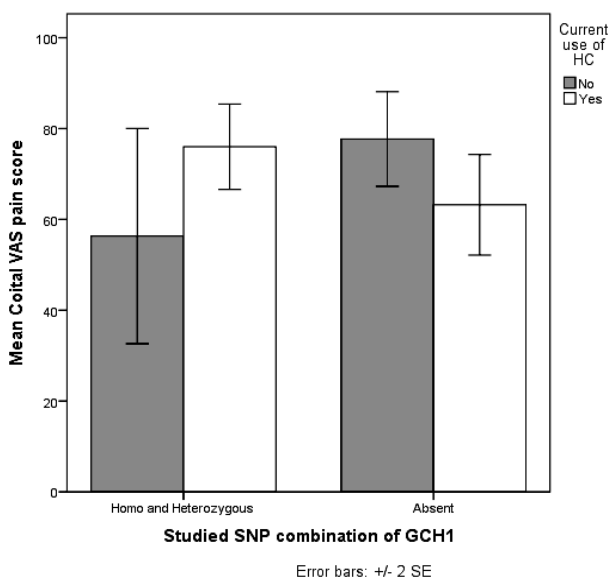


Figure 12. Interaction effect of the studied SNP combination for the *GCH1* gene and use of hormonal contraceptives (HCs) on coital pain scores among patients currently receiving treatment (n=38). Carriers of the minor haplotype not using HCs had lower coital VAS pain scores than carriers of the major haplotype. VAS= visual analogue scale

To further explore the association between the SNP combination and HC use in relation to the other measures of pain, a series of GLMs including PPTs on the arm, on the leg, in vestibular areas A and B, and bodily pain scores were performed. There were no associations in either the total patient group or the total patient plus control group. However, separate analysis of women

with PVD receiving current treatment showed a relationship between the *GCH1*-SNP combination, use of HCs, and PPTs on the arm, as well as a borderline significant relationship to PPTs on the leg.

The relationship between the *GCH1*-SNP combination, use of HCs, and PPTs on the arm is shown in Figure 13. Among women with PVD currently receiving treatment and not using HCs (n = 23), pain sensitivity on the arm was lower (higher PPTs) in carriers of the *GCH1*-SNP combination than in non-carriers. Among women with PVD receiving therapy and also using HCs (n = 15), the picture was inversed; pain sensitivity was higher (lower PPTs) in carriers than in non-carriers.

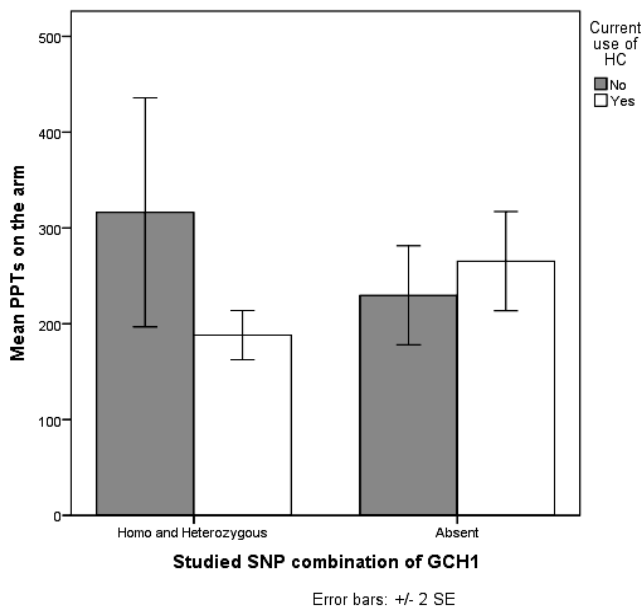


Figure 13. Interaction effect of the studied SNP combination for the *GCH1* gene and use of hormonal contraceptives (HCs) on pressure pain thresholds (PPTs) on the arm among patients currently receiving treatment (n=38).

9.7.4 *OPRM1* polymorphism and PVD

The rare 118G allele was significantly more common in controls than in patients. The probability of having PVD was almost two times higher for participants who were homozygous for the 118A allele compared to participants who were hetero- or homozygous for the 118G allele (OR = 1.846, CI: 1.03–3.31, p = 0.039).

9.7.5 *OPRM1* polymorphism and pain sensitivity

PPTs on the leg were higher in participants carrying the 118G genotype than in participants carrying the 118A genotype; see Figure 14. There were no differences in the other pain

measurements between carriers of the 118G and 118A genotypes in both groups combined. When patients and controls were analyzed separately, PPTs on the leg and arm were significantly higher in controls carrying the 118G genotype than in those carrying 118A. There were no significant differences in the patients group.

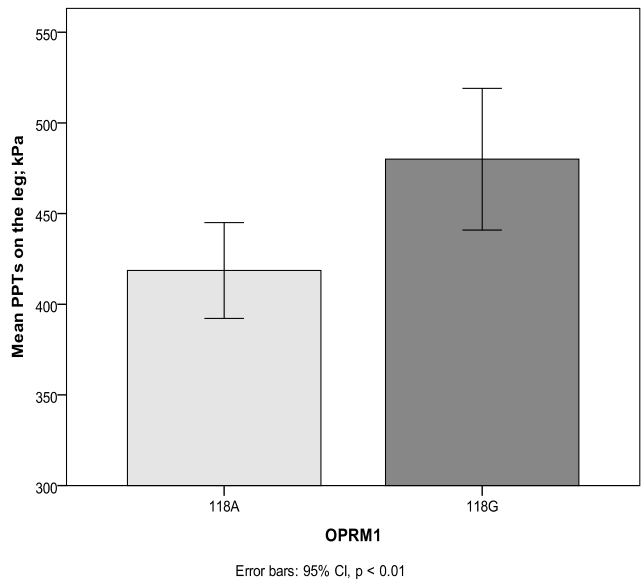


Figure 14. Lower pain sensitivity (higher pressure pain thresholds) seen in carriers of the 118G-allele of the *OPRM1*-gene. PPT= pressure pain threshold.

9.7.6 β-endorphin, PVD, *OPRM1*, and pain sensitivity

Plasma levels of β-endorphin were significantly higher in patients (mean 17.9 fmol/ml, SD 4.71, n = 80) than in controls (mean 15.8 fmol/ml, SD 4.03, n = 95; z = -3.61, p < 0.001); see Figure 15. Mean levels of β-endorphin were lower in carriers of the 118G genotype (mean 16.0 fmol/ml, SD 4.26, n = 64) than in carriers of the 118A genotype (mean 17.2 fmol/ml, SD 4.55, n = 111), with a tendency toward a significant difference (z = 1.92, p = 0.055).

There was a significant correlation between plasma levels of β-endorphin and the pain score (rho = 184, p = 0.015), with higher levels of β-endorphin among participants with more concomitant pain disorders. There were no significant correlations between plasma levels of β-endorphin and PPTs or the coital VAS score. Further, there were no interaction effects between β-endorphin levels and gene variants on the pain measurements.

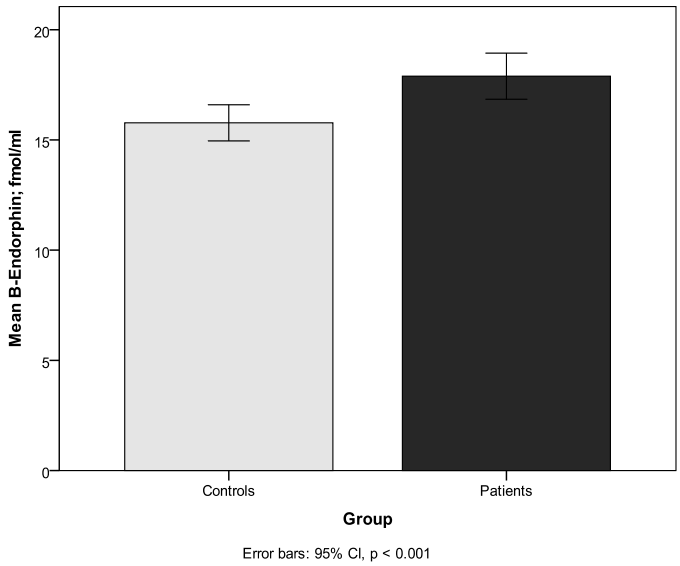


Figure 15. Higher plasma concentrations of β-endorphin in patients compared to controls.

9.7.7 *5HT-2A* polymorphism and PVD

The AA and TT genotypes were significantly more common in controls than in patients. The probability of having PVD was nearly three times higher for participants who were homo- or heterozygous for the G or C alleles compared to participants who were homozygous for the A or T alleles (OR = 2.9, CI: 1.2-6.9, p = 0.017).

9.7.8 HADS scores, *5HT-2A* polymorphism, and PVD

The probability of having PVD was five times higher for participants with a HADS anxiety score ≥ 8, compared to participants with a lower score (OR= 5.2, CI: 2.8-9.5, p < 0.001). In addition, in a multivariate model that included both genotype and HADS anxiety score, the ORs were even higher (genotype: OR 3.5, CI: 1.3-8.9, p = 0.01; HADS anxiety score: OR 5.6, CI: 2.9-10.6, p < 0.001).

However, there were no significant differences in proportions of HADS scores <8/≥ 8 between carriers of the different genotypes in the whole group. The frequency of a HADS anxiety score ≥ 8 was the same (38%) in the AA carriers (11 of 29) as in the AG/GG carriers (66 of 172), while the frequencies of depression were 3% (1 of 29) and 6% (10 of 172), respectively. There were no interaction effects between *5HT-2A* polymorphism, patient versus control status, and HADS scores.

9.7.9 *5HT-2A* polymorphism, HADS scores, and pain sensitivity

The bodily pain scores in participants who were homo- or heterozygous for the G allele were higher than those in participants who were homozygous for the A allele. No other allele-linked

differences in the pain measurements were found in the whole population or when patients and controls were analyzed separately. Multivariate analysis found no interaction effects of *5HT-2A* polymorphism and patients versus controls with respect to the pain measurements. Similarly, there was a significantly higher mean bodily pain score among participants with a HADS score indicating anxiety (≥8) but no differences in the other pain measurements. There were no significant differences in pain measurements when patients were grouped according to HADS depression score, nor was there any interaction effect between *5HT-2A* polymorphism and HADS scores with respect to pain measurements or a PVD diagnosis. However, as shown in Figure 16 there was a significant interaction effect for patients versus controls and HADS anxiety score above or below 8 with respect to the bodily pain score ($F = 4.933$, $p = 0.028$). In the control group, participants with a HADS anxiety score ≥8 had a higher bodily pain score than that in participants with a score <8, whereas there were no differences in the patient group.

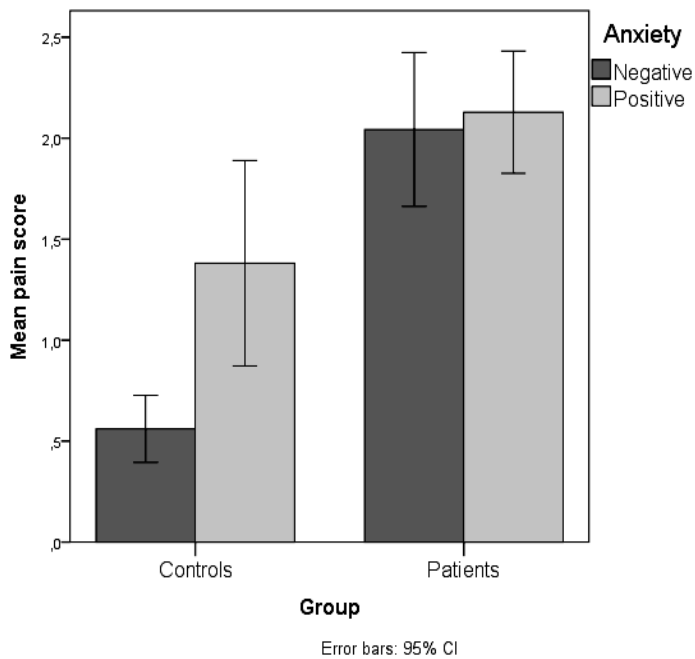


Figure 16. A significantly higher mean bodily pain score was seen among controls with a HADS score for anxiety ≥ 8 compared to those with a lower score, whereas among patients no difference was seen.

10 DISCUSSION

10.1 DISCUSSION OF MATERIALS AND METHODS

10.1.1 Participants

10.1.1.1 Patients

One of the strengths of the studies in this thesis was the very well defined patient group; all the patients except 6 were initially diagnosed by the same three physicians at the same open care clinic. An accurate diagnosis and a clear definition of the inclusion criteria are essential if studies are to be compared or reproduced. In these studies, all the patients were examined again when measuring the vestibular PPTs to confirm the absence of clinical signs of infection or dermatoses. The intermittent character of the pain in this condition is an important feature that differentiates it from many other chronic pain conditions, for example non-provoked vulvodynia. All patients but one had a diagnosis of provoked pain only; the outlying patient was diagnosed with generalized vulvodynia and was subsequently excluded from the studies.

One issue of concern is the fact that the initial response rate to enquiries regarding interest in participating in the studies was low, resulting in a risk of a selection bias in the patient group. The low response rate could have been the result of many factors, including the young age of the patients, which is likely to lead to frequent changes in life circumstances, including moving away from the Stockholm area. Nevertheless, there was a considerable range of responses to the tested parameters (pain sensitivity, treatment outcome, etc.) in all studies, indicating that the patient group was not homogeneous and it is not likely that the genetic results was affected by the response rate. Since the primary aim of Study I was to identify predictors of treatment outcome and not to evaluate the different treatment modalities per se, the study population can still be considered as reliable also in this work.

The participants were treated sometime between 1997 and 2008 while the study was carried out between 2008 and 2010, which could have increased the risk of recall bias. On the other hand, long-term follow-up studies after treatment for PVD are uncommon, and the median follow-up time of 5 years for these patients has provided some interesting data.

Although the study population could be considered fairly large for a study in the field of PVD, genetic studies require very large study groups to render reliable results and an even larger study population might have been favorable. However, the initial sample size of 100 participants in each group was estimated to give sufficient power to detect a possible difference in SNP frequencies between patients and controls, according to a method for optimizing sample sizes in candidate gene studies described by Belfer et al. [180] based on the frequency of the minor allele.

10.1.1.2 Controls

In a case-control study, the choice of the control group is equally important in order to avoid a control bias decreasing the external validity of the results. Regular use of analgesic and antidepressive drugs was an exclusion criterion for the controls, and this could have resulted in

a risk of the control group having lower pain sensitivity and less depression than the normal population.

10.1.2 **Questionnaires**

We choose to use study-specific questionnaires to survey pain co-morbidity and other parameters in preference to validated questionnaires such as the McGill Pain questionnaire or SF-36. This decision was made because questionnaires like the McGill are very extensive while in this study we were interested in a number of specific pain disorders. The creation of a bodily pain score has not been validated for women with vulvar pain but has been used in an earlier study by Granot et al.[84]. The participants were asked to report other frequent pain problems without defining the word frequent or the intensity of the pain. This could have resulted in inter-individual differences. It is also possible that it would have been of value to include other pain disorders (for example, interstitial cystitis [181] and orofacial pain [40], which appear to be associated with PVD) in the analyses. There were no reports of bladder pain and only one report of orofacial pain in response to our questionnaire, but it is possible that asking for defined pain modalities instead of using an open question might have yielded different answers. Dysmenorrhea was not included in the pain score because the use of HCs varied among the participants and HCs can influence the frequency and intensity of menstrual pain. The HADS is a validated instrument, modified for Swedish use.

10.1.3 **Pain measurements**

The fact that all participants were examined in the same menstrual phase, by the same examiner, in a blinded fashion, strengthens the study results. Pain sensitivity varies during the menstrual cycle and this method was chosen to standardize for any such differences [125]. Both peripheral and vestibular PPTs were measured using previously described, commonly used procedures and instruments [38, 179]. Every effort was made to standardize the procedures and to increase the exerted pressure at a constant rate. Nevertheless, the wide inter-individual variation in PPTs could reflect methodological inconsistencies as well as differences in pain sensitivity. Methods where participants orally report pain thresholds must inevitably include the participants' reaction times, but this method was chosen for practical reasons and to replicate previous studies so that results could be compared.

10.1.4 **Candidate genes**

Candidate gene association studies are associated with their own inherent difficulties; previous results have been inconsistent and difficult to reproduce. One of the advantages of association studies is the expected improved power for detection of small to moderate genetic effects, as one can select unrelated subjects to optimize the clinical phenotype. Belfer et al. suggested a method of prioritizing candidate genes and polymorphisms in association studies of pain, where each candidate gene is rated according to: 1, the strength of evidence supporting involvement of the gene in pain processing; 2, the frequency of the specific variant; and 3, the likelihood that the polymorphism will alter function [180]. The *GCH1*, *OPRM1* and *5HT-2A* SNPs that were investigated in Study II-IV all rate high according to this method. There is much scientific evidence supporting the role of these three genes in endogenous pain modulation and the rare variants have frequencies varying from 20 to 40%.

We were also cognizant of the fact that, although the number of women seeking medical care for PVD has increased in recent years, it is still a limited patient category and to collect the many thousands of patients needed for GWS is not particularly feasible. Since the diagnosis is clinical, with no available laboratory or histological tests for confirmation, maintenance of accurate diagnoses in a multicenter study might also be difficult.

10.1.5 Statistics

Study I was exploratory in nature; correlation with treatment outcomes was tested for several factors, which increased the risk of a false positive association (type 1 error = rejecting the null hypothesis of no correlation when it is actually true). However, the results of several multivariate logistic regression analyses indicated that the number of concomitant bodily pain was linked to both treatment outcomes and the intensity of current coital pain, which strengthens the validity of the results.

In Studies II-IV, the relationship between the studied polymorphisms and PVD was hypothesis driven; that is, a clearly defined hypothesis with clear definitions of predictor (SNP) and outcome (PVD) was decided on before the study was conducted, thus reducing the number of statistical tests required, and subsequently the risk of a type 1 error. Studies with small sample sizes, wide variability in the measured parameters, or a small true effect have low statistical power and the chance of missing a true difference (type 2 error = accepting the null hypothesis when it is actually false) is increased. When correlating the polymorphisms with the pain measurements, we divided the material into subgroups; this increased the risk of a type 2 error, and the apparent lack of correlations between genetic polymorphisms and pain measurements must therefore be interpreted with some caution [182].

10.2 DISCUSSION OF THE RESULTS

10.2.1 Background data

The controls were significantly younger than the patients. Although gene expression (epigenetic changes) can change with ageing [183], the mean age difference of 5 years is unlikely to affect the results for pain measurements or genetic polymorphisms. The age difference could, however, explain the differences in occupation, duration of use of HCs and birth rate between the groups. The ethnicity of the groups did not differ, which minimizes bias due to gene-race interactions.

10.2.2 Pain co-morbidity, HADS results, and pain sensitivity

Our results robustly confirm previous results indicating more frequent occurrence of other bodily pain symptoms, higher levels of anxiety and depression, and higher general pain sensitivity in women with PVD than in healthy controls [33, 37, 38, 44-47, 84]. Using coital pain ratings excludes women not engaging in vaginal intercourse but since only five participants in this studies failed to report coital pain due to beeing apareunic we consider coital pain as a reliable measure in this setting. The tampon test is a way to determine PVD treatment outcome and current pain intensity also among apareunic women and it might therefore be considered as a preferable method, however, this method has other disadvantages since not all women with

PVD experience pain at tampon insertion and the sexual functioning is not included in the testing.

10.2.3 **Treatment outcomes**

The patients had received multifaceted, multidisciplinary treatment with different combinations of therapeutic approaches, according to their specific needs. The duration of treatment was also individualized. Desensitizing local anesthetic gel (lidocaine 2%) was by far the most common treatment; in most cases this was used in combination with pelvic floor muscle rehabilitation. Twenty-eight participants received more comprehensive treatment involving psychosexual counseling based on CBT for approximately one year, with successively less frequent sessions. The treatment outcome assessment used in this study was based on subjective self-reporting and could therefore be influenced by retrospective recall errors as well as by factors occurring after the completed treatment. Comparisons of the outcomes of different treatments were not made because of the varying durations of follow-up, the individualized management of the participants with a lack of randomization, and the lack of pre-treatment values for coital pain.

The results indicate higher rates of an incomplete response to treatment in women with primary PVD than in those with secondary PVD, which is consistent with earlier results [84]. In our study, 31% of patients with primary PVD reported major improvement or complete recovery, which is in line with 30-50% improvement in PVD symptoms in patients treated with placebo in other studies [77, 78] and suggests that the treatments used are less effective in this subgroup. Similarly, two independent studies have reported higher incomplete response rates to surgery in women with primary PVD than in those with secondary PVD [75, 85]. It still needs to be clarified whether the etiology is different for primary and secondary PVD and whether specific therapies are needed for these subgroups.

10.2.3.1 Evaluation of treatment outcome

Many patients describe the posterior part of the vestibule (area B) as the most painful during intercourse. In this study we used three different parameters for assessing vestibular pain and dyspareunia. While the vestibular PPTs increased in area B after completed treatment, there was only poor correlation between vestibular PPTs and the patients' subjective evaluations of their PVD status (no pain to severe pain during vaginal intercourse). In contrast, there was a strong correlation between coital pain assessed using a VAS and the patients' subjective evaluations of current PVD status. Among clinicians, the cotton-tip test is often used for both diagnosis and for re-testing patients after treatment. The use of vulvar algesiometers in our studies was an attempt to be more objective in measuring vestibular pain sensitivity. However, the results suggest that using a VAS provides a better indication of how the patients experience actual pain during intercourse. This supports the suggestion in a recent review [64] that patient self-ratings of vestibular pain on a numeric scale might be a superior method for evaluation of treatment outcomes.

10.2.4 **Predictors of treatment outcome**

Of the medical factors included in this study, we found that the number of other bodily pain conditions was the variable most strongly associated with treatment outcome. The bodily pain score was intended to provide a method of analyzing whether the number of other concomitant pain disorders was more relevant to treatment outcomes than a specific pain modality. According to our results, women with fewer other pain disorders are likely to respond better to treatment than those with multiple other pain disorders, thus verifying previous findings [84]. An increased number of concomitant pain disorders could indicate more pronounced general pain hypersensitivity, which suggests differences in endogenous pain modulation as a possible explanation for this result. It has previously been shown that women who had completed treatment for PVD still had lower PPTs on the arm and leg than healthy women, regardless of the treatment outcome [68]. Nonetheless, in this study, the peripheral PPTs did not correlate significantly with whether the participants improved. This seemingly contradictory finding might be a reflection of long-standing pain disorders and mechanical PPTs being influenced by different aspects of endogenous pain modulation. Another explanation might be differences in psychological traits such as fear and avoidance of pain, catastrophizing, and lack of pain self-efficacy. These traits have been shown to predict treatment outcome in an earlier study [83] and have been associated with other chronic pain conditions, although the exact mechanism of interaction is not known. A more specific evaluation of the participants' psychosexual function and distress was not carried out in this study, although we found no association between treatment outcome and the HADS scores for anxiety and depression.

10.2.5 **Genetic findings**

10.2.5.1 Genetic contribution to the pathogenesis of PVD

The main aim of these studies was to investigate the possible contribution of polymorphism in certain genes involved in endogenous pain modulation to the risk of developing PVD and to the general pain hypersensitivity seen in these women. We found a higher probability of being diagnosed with PVD among carriers of the 118A genotype of the *OPRM1* gene (OR 1.8) and the 102C genotype of the *5HT-2A* gene (OR 2.9). However, we found no association between a PVD diagnosis and the studied haplotype in the *GCH1* gene. The results indicate the involvement of both opioid and serotonin systems in the pathogenesis of PVD. The involvement of these systems in human pain modulation is well documented, and the studied polymorphisms have been shown in several studies to affect pain sensitivity, although there remains some disagreement on their exact mechanisms and effects. Nevertheless, the impact of a single gene polymorphism on the complex phenomenon of chronic pain can be expected to be only modest, and further studies investigating possible gene-gene and gene-environment interactions would be of value.

The studied SNP combination in the *GCH1* gene have been found to occur with a frequency of approximately 15% in a normal Caucasian population of mixed sexes [148]. We found a carrier frequency for this SNP combination (homo- and heterozygous) in study II of approximately 28% in our population of young Swedish women (patients and controls combined), see Table 7. However, this frequency is in line with previous findings in Swedish females [155].

The carrier frequencies of the different alleles of the *OPRM1* and *5HT-2A* genes found in Study III and IV were similar to previous findings, see Table 8 and 9 [160, 168].

Both the opioid and the serotonin systems have direct effects on pain signaling and modulation, peripherally and centrally, and they also affect psychological pain modulators. The endogenous opioid system and β-endorphin play a role in anxiety, stress response and sexual behavior [135]. Investigation of the possible effects of the A118G polymorphism on the activation of the hypothalamus pituitary adrenal axis and cortisol release found higher cortisol concentrations at baseline and after naloxone infusion among 118G carriers [184]. Also, morning awakening cortisol levels appear to be blunted in women with PVD, indicating chronic stress [185]. The serotonergic systems´ contribution to mood disorders is a well known fact, proven by the efficacy of treating these disorders with selective serotonin reuptake inhibitors (SSRIs) [167]. Furthermore, SSRI treatment has a negative effect on sexual desire and function. Our results verify previous findings of a higher frequency of anxiety mood disorder in PVD patients than in healthy controls. The association between a high HADS anxiety score and PVD was even stronger than the association with the *5HT-2A* polymorphism (OR 5.2). It is therefore interesting to speculate whether the differences in μ-opioid and serotonin receptor polymorphism between patients and controls might partly explain the previously mentioned differences in psychological traits as well as differences in pain sensitivity.

10.2.5.2 GCH1 gene and HC interaction

The lack of association between the studied *GCH1* SNPs and PVD indicates that the observed pain in PVD might be regulated by aspects of endogenous pain modulation that do not involve the BH4 pathway. Our findings are in line with the previously reported lack of association between variations in *GCH1* and chronic widespread pain, a predominantly female, long-standing pain condition which shares over-lapping features with PVD [153]. The most robust associations between *GCH1* and pain responses have been noticed in acute inflammatory pain models [147, 154]. However, in our study, there was a lack of correlation between the studied SNP combination and pressure pain sensitivity not only in non-sensitized skin areas on the arm and leg but also in the sensitized vestibular mucosa. This might suggest a modality-specific effect of *GCH1* variations on pain.

Nevertheless, we found a gene-hormonal interaction of *GCH1* polymorphism and use of HCs in relation to pain sensitivity. Patients with current treatment reported higher coital VAS pain score as compared to patients with completed treatment and therefore it was anticipated that the hypothesis of an association would be more evident in this group. We therefore analyzed patients currently receiving treatment as a group and found a correlation between the studied SNP combinations and lower pain scores in patients not using HCs. Interestingly, in patients using HCs, the relationship was inversed; pain sensitivity was greater in carriers of the SNP combination. It is inviting to speculate that the fact that a subgroup of women with PVD are improved or even cured when HC use is terminated [26] could result from the influence of genetic differences on endogenous pain modulation. According to our findings, it appears possible that PVD patients carrying the studied SNP combination would benefit the most from this intervention. However, it has also been shown that HCs could have a direct effect on the

vestibular mucosa [88, 93] and it is not clear whether the higher coital pain ratings seen among HC users is caused by morphological changes, by hormonal effects on endogenous pain modulation, or by an interaction of both. If the mucosal effects of HCs are greater than the pain modulatory effect, it could explain the lack of a pain-protective effect from the studied *GCH1*-SNP combination among users of HCs. We also found a relationship between the *GCH1*-SNP combination, use of HCs, and PPTs on the arm, which strengthens the idea of an interactive effect of these variables.

In a gene-environment study using the candidate gene approach, such as the gene-sex hormonal interaction investigated in this study (Study II), both genetic and environmental variables are hypothesized a priori. This creates a large number of testable hypotheses and increases the risk of a type 1 error, i.e. finding a false positive. The power to detect interactions is typically lower than to detect main effects and therefore the result need to be interpreted with some caution. [186]

10.2.5.3 OPRM1 polymorphism associated with pain sensitivity

PPTs were higher on the leg in carriers of the 118G allele in the *OPRM1* gene. However, when analyzed separately, the findings were only consistent among controls. The healthy women had higher PPTs on the leg as well as on the arm, but there were no significant differences in patients. Although many studies have investigated the pain modulatory effects of the A118G SNP in the μ-opioid receptor, the results have been somewhat conflicting. Our findings are in line with previous results that indicated a pain-protective effect of the 118G allele [158, 159], but contradict the findings of Huang et al., who found no statistically significant allele-linked differences in PPTs in healthy women [160]. The initial finding of higher PPTs among 118G carriers noted by Fillingim et al. was most evident in men, and several later studies showed a sex-genotype interaction where, in contrast to our results, women with the 118G allele were more sensitive to pain than were those with the 118A allele [162, 163]. However, these findings were linked with clinical pain and pain after surgery rather than experimental PPTs and the pain modality might have affected the result. The fact that only one of the measured PPTs was associated with the genotype and that the association was only consistent in the control group raises questions. There could be several explanations for this. Controls had higher PPTs than patients, which means that a possible association could be more evident in this group. Subdividing the results could also have reduced the power of detecting an association. Apart from these factors, the impact of a single gene polymorphism on the general pain hypersensitivity seen in patients with PVD is expected to be modest and therefore difficult to establish statistically.

10.2.5.4 5HT-2A polymorphism and anxiety associated with bodily pain

There was a correlation between concomitant bodily pain scores and the *5HT-2A* SNPs; there were more pain symptoms among G/C carriers but no allele-linked differences in the other pain measurements. It is possible that the bodily pain score reflects general pain hypersensitivity, which is different from the sensitivity measured by experimental PPTs. The result is concordant with the association of the CC genotype with other predominantly female, generalized pain syndromes such as fibromyalgia and CWP [170]. A higher bodily pain score was also reported

by participants with a HADS anxiety score ≥ 8. However, there was no association between the SNPs and the HADS scores and no interactive effect of *5HT-2A* polymorphism and anxiety on patients versus controls or pain measurements, suggesting that the associations are independent of each other. Additionally, when analyzed separately, the correlation between a HADS anxiety score ≥8 and a higher bodily pain score was only consistent among controls. The lack of difference in bodily pain score between patients with a HADS anxiety score above or below 8 indicates that the amount of concomitant pain was not correlated with the level of anxiety in this group.

10.2.6 **Clinical implications**

Our results indicate that several genetic polymorphisms affect endogenous pain modulation and possibly contribute to the risk of developing PVD. However, pain is a very complex phenomenon and a single genetic polymorphism can only be expected to contribute to a limited extent to a chronic pain syndrome. The sensation of pain is subjective and is affected by several factors in addition to congenital endogenous pain sensitivity, such as personality traits, previous experience, and co-morbidity. Nevertheless, our results contribute to the understanding of this challenging condition. Taken together, the results of this research strengthen the conceptualization of PVD as a general pain condition. A careful medical history to investigate the degree of other concomitant pain disorders and the subgroup of PVD is proposed as a means of identifying patients who might need a higher level of care and who could benefit from a referral to a specialist vulvar care or pain unit. The age of onset of PVD is usually between 18 and 25 years; general pain hypersensitivity is already present at this age, but rarely causes disability. We believe that early recognition and treatment, with the risk of further development of chronic pain taken into consideration, might prevent aggravated pain problems in this patient group in addition to restoring their sexual health.

10.2.7 **Future perspectives**

It remains for continuing research to fully elucidate the pain mechanisms involved in PVD and other chronic pain conditions so as to improve treatment. The results of this thesis point towards a value of exploring the biomedical mechanisms of the involvement of the serotonin system in chronic pain disorders in more depth as a possible target for future pharmacological treatment. More studies are also needed to clarify the suggested differences in clinical presentation and etiology between primary and secondary PVD, in order to tailor specific treatments and optimize outcomes in the different subgroups.

More randomized, blinded treatment studies are also warranted, with an international consensus on how best to evaluate treatment outcomes.

Ultimately, the optimal goal of continuing investigations of the biomedical and psychosocial mechanisms behind this life-quality diminishing disorder is to eventually be able to prevent the vestibular pain.

11 CONCLUSIONS

Main conclusion:

The main results indicate the involvement of both the opioid and the serotonin systems in the pathogenesis of PVD, with higher probability of being diagnosed with PVD in carriers of the 118A genotype of the *OPRM1* gene (OR 1.8) and the 102C genotype of the *5HT-2A* gene (OR 2.9). No association was found between a PVD diagnosis and the studied polymorphism in the *GCH1* gene.

Further study-specific findings:

I. A successful treatment outcome was more likely in PVD patients with fewer other concomitant pain disorders. The number of other pain disorders was also associated with the intensity of coital pain. The results also indicated higher rates of incomplete response to treatment in women with primary PVD than in those with secondary PVD.

II. Among patients currently receiving treatment for PVD, the studied polymorphism of the *GCH1* gene and use of HCs had an interaction effect with respect to pain sensitivity. PVD patients carrying the studied genotype and using HCs had lower PPTs than non-carriers.

III. Increased pain sensitivity with lower PPTs was found in participants carrying the 118A genotype of the *OPRM1* gene. Levels of plasma β-endorphin were higher in PVD patients than in controls. General pain sensitivity was greater and there was more concomitant bodily pain in PVD patients than in controls.

IV. Symptoms of anxiety were more common in PVD patients than in controls, with an OR of 5.2 for a PVD diagnosis among participants with heightened anxiety symptoms. There was a correlation between more concomitant bodily pain and the 102C genotype of the *5HT-2A* gene as well as with anxiety symptoms.

12 POPULÄRVETENSKAPLIG SAMMANFATTNING

En studie i smärtgenetik och samsjuklighet i andra smärttillstånd hos kvinnor med vestibulit och friska kontroller.

Bakgrund

Samlagssmärta är ett vanligt hälsoproblem. Förekomsten är osäker men flera studier har visat att ca.10-15% av unga kvinnor drabbas. Den vanligaste orsaken till samlagssmärta bland unga kvinnor är ett tillstånd kallat vestibulit (också kallat provocerad vestibulodyni). Vestibulit är ett långdraget smärttillstånd som kännetecknas av intensiv smärta vid beröring av området kring slidöppningen och försök till vaginal penetration. Smärtan medför att de drabbade kvinnorna inte kan ha ett normalt sexuellt samliv, vilket får stora konsekvenser för deras allmänna välbefinnande och partnerrelation. Orsakerna till vestibulit är ännu inte helt klarlagda. Troligen är det en samverkan av både kroppsliga faktorer, som till exempel infektioner och hormonell påverkan, och psykosexuella faktorer. Kvinnor med vestibulit uppger ofta smärta även från andra delar av kroppen som muskelvärk, huvudvärk samt problem från mag-tarmkanalen. Det är visat att dessa kvinnor har ett ökat antal ytliga nervfibrer i vävnaden kring slidmynningen och att dessa nerver har ökad smärtkänslighet. Kvinnor med vestibulit har även sänkta smärttrösklar på andra delar av kroppen jämfört med friska kvinnor och det finns en koppling till andra kroniska smärttillstånd som t.ex. fibromyalgi.

Under senare år har kunskaperna inom smärtgenetik gått framåt. Flera gener har identifierats som påverkar kroppens smärtkänslighet och risken att utveckla ett kroniskt smärttillstånd bl.a. gener inblandade i bildandet av signalsubstanser i nervsystemet (GCH1), i kroppens känslighet för kroppseget endorfin (OPRM1) och i serotoninsystemet (5HT-2A). Om dessa genetiska faktorer kan bidra till utvecklandet av vestibulit har dock inte tidigare studerats.

Utbudet av effektiva behandlingsmetoder är fortfarande begränsat. För närvarande används oftast en kombination av lokal smärtbehandling med bedövningsmedel, bäckenbottenavslappnande övningar och kognitiv beteendeterapi. Behandlingen är ofta långvarig och behandlingsresultatet är, om än i de flesta fall gott, varierande. En del av de nuvarande behandlingsmetoderna är mycket resurskrävande och det är viktigt att det sker en fortsatt utveckling inom detta område. För att kunna hitta nya, effektivare behandlingsmetoder måste vi lära oss mer om de utlösande orsakerna till vestibulit.

Vetenskaplig huvudmålsättning:

- Vår huvudmålsättning var att kartlägga förändringar i tre gener som har en känd effekt på kroppens smärtreglering (GCH1-, OPRM1- och 5HT-2A- generna) hos kvinnor med vestibulit och friska kontroller och dessa genförändringars möjliga bidragande effekt till en ökad risk att utveckla vestibulit. (Delarbete II-IV)

Övriga delmål var:

- Att undersöka om det finns faktorer som kan förutse behandlingsresultatet samt kartlägga samsjuklighet med andra smärttillstånd hos kvinnor med vestibulit. (Delarbete I)
- Att undersöka en möjlig kombinationseffekt av förändringar i GCH1-genen och p-pilleranvädning på smärtkänslighet hos kvinnor med vestibulit och friska kontroller (Delarbete II)
- Att undersöka en möjlig korrelation mellan förändringar i OPRM1-genen och halterna av β-endorfin i blodet och smärtkänslighet hos kvinnor med vestibulit och friska kontroller.(Delarbete III)
- Att undersöka en möjlig samverkan mellan förändringar i 5HT-2A-genen, ångest- och depressionssymtom, smärtkänslighet och risken att utveckla vestibulit (Delarbete IV)

Material och metoder

Studien utfördes mellan maj 2008 och maj 2010. Sammanlagt deltog 109 kvinnor med vestibulit, tidigare eller nuvarande patienter vid vulvamottagningen på Danderyds sjukhus, och 103 friska kontroller i samma åldergrupp.

Alla deltagare svarade på frågeformulär där bland annat samtidiga smärtsymtom kartlades och samlagssmärta självskattades med hjälp av en visuell analog skala från 0-100. Därutöver utfördes screening avseende ångest och depression. Alla utom 11 patienter genomgick mätning av smärttrösklar för trycksmärta på arm och ben samt lämnade blodprov för de genetiska analyserna. Patienterna genomgick även mätning av smärttrösklar för trycksmärta kring slidmynningen. Alla mätningar utfördes under samma fas i menscykeln och undersökaren kände inte till om personen ifråga var patient eller kontroll (utom vid mätningarna i vestibulum).
Blodprovsanalyserna utfördes på Centrum för farmakologisk biovetenskap samt Genomcenter i Uppsala där frekvenserna av vissa kända förändringar i de ovan nämnda generna kartlades och halterna av endorfin mättes.

Resultat

- Den enskilda faktor som starkast var kopplat till behandlingsresultat var förekomst av annan smärta. Det var troligare att vestibulitpatienter med högst en annan smärta blev "mycket bättre" eller "helt bra" jämfört med patienter med fyra eller fler andra smärtor. Hur många andra smärtor patienten hade var också kopplat till graden av samlagssmärta. (Delarbete I).
- Vi fann inget samband mellan den GCH1-variant som tidigare definierats som smärtskyddande och vestibulit. Inte heller fann vi något samband mellan denna genvariant och känslighet för trycksmärta, grad av samlagssmärta eller förekomst av

annan smärta. Däremot fann vi att vestibulitpatienter som var bärare av den definierade genvarianten och använde ett hormonellt preventivmedel hade högre smärtkänslighet jämfört med icke bärare. Detta resultat visar på en möjlig förklaring till att en del kvinnor med vestibulit förbättras efter att de slutar använda hormonella preventivmedel (Delarbete II).

- Vi fann ett samband mellan en viss variant av OPRM1-genen och vestibulit samt känslighet för trycksmärta. De deltagare som var bärare av den ovanligare varianten hade mer sällan vestibulit och var mindre smärtkänsliga jämfört med dem som var bärare av den vanliga varianten. Patienterna hade högre halter av β -endorfin jämfört med kontrollerna. Dessa resultat tyder på att skillnader i kroppens smärtreglering som involverar det opioida systemet kan bidra till risken att utveckla vestibulit och till den ökade smärtkänsligheten bland dessa patienter (Delarbete III).
- Vi fann också ett samband mellan en viss variant av 5HT-2A-genen och vestibulit samt att patienterna uppgav väsentligt högre ångestnivåer jämfört med kontrollerna. Däremot fann vi inget samband mellan denna genförändring och graden av ångest. Annan smärta var vanligare bland deltagare som var bärare av genvarianten och uppgav höga ångestnivåer. Resultaten upprepar tidigare fynd av ökad förekomst av ångest bland kvinnor med vestibulit och stämmer väl överens med ett sedan tidigare känt samband mellan denna genförändring och fibromyalgi. Detta erbjuder en möjlig förklaring till likheterna mellan vestibulit och fibromyalgi och stärker intrycket av vestibulit som en del i ett generellt smärtsyndrom (Delarbete IV).

Slutsats

Våra resultat visar på att flera genetiska förändringar som påverkar kroppens smärtkänslighet och kan bidra till utvecklandet av vestibulit. Dock är smärta ett väldigt komplext fenomen och en enskild genetisk förändring kan bara förväntas bidra med en liten del av förklaringen bakom långdragen smärta. Upplevelsen av smärta är subjektiv och påverkas av många faktorer utöver medfödd smärtkänslighet som t.ex. tidigare erfarenheter, personlighet och annan sjuklighet. Våra studier kan ändå bidra till den övergripande förståelsen av detta svårbehandlade tillstånd. Sammantaget stärker våra resultat uppfattningen att vestibulit inte är ett isolerat fenomen utan en del av ett generellt smärtöverkänslighetssyndrom. Eftersom kvinnor som drabbas av vestibulit ofta är unga är detta viktigt att tänka på i omhändertagandet av dessa patienter. En tidig korrekt diagnos och en effektiv behandling kan bidra inte bara till att dessa kvinnor återfår sin sexuella hälsa utan även till att undvika framtida förvärrade smärtproblem.

13 APPENDIX

13.1 QUESTIONNAIRE

Date:
Code No.…..
Age.....
Occupation..... Studying Employed Unemployed

PREVIOUS AND PRESENT DISEASES

- Are you diagnosed with any medical or psychiatric desease? No Yes Year
  If yes: What treatment have you received?
- Have you ever had surgery? No Yes
  If yes: Type of surgery?
- Regular medications? No Yes
  If yes: Name of medicine?
- Do you currently receive any pain treatment? No Yes
  If yes: Name of treatment/medicine?
- Do you have any dermatological disease? No Previously Now
  If yes: What diagnosis?
- If current treatment? What medication?
- Have you ever received treatment for depression? No Previously Now
- Have you ever had a professional consultation as treatment for depression? No Previously Now

FREQUENT PROBLEMS WITH:

- Headache (tension)? No Previously Now
- Migraine? No Previously Now
- Muscle pain? No Previously Now
- Gastritis? No Previously Now
- Irritable bowel? No Previously Now
- Constipation? No Previously Now
- Back pain? No Previously Now
- Any other pain problem? No Previously Now
- If yes: What problem?

ALLERGIES

Do you have: Treatment

- Conjunctivitis? No Previous Now
- Rhinitis? No Previous Now
- Eczema? No Previous Now
- Asthma? No Previous Now
- Food allergy? No Previous Now
  If yes: What food?
- Allergy to medication? No Previous Now

If yes: What medication?

GYNECOLOGY

Menstruation

- Do you have regular periods? No Yes
- Do you have dysmenorrhoea? No Yes
  If yes: Analgesic medication? No Yes
  If yes: Name of medication.....
- First day of last period?.....……..

Pregnancies

Have you:

- Been pregnant? No Yes Number.....
- Given birth? No Yes Number.....
- Had a miscarriage? No Yes Number.....
- Had a legal abortion? No Yes Number.....

Birth control

- Have you ever used oral contraceptives? No Yes
  If yes: Age when you first started?
- Are you currently using oral contraceptives?
  If yes: Name of pills? No Yes
- Total time you´ve been taking oral contraceptives?

13.2 **PATIENT-SPECIFIC QUESTIONNAIRE**

- How long have you experienced pain during intercourse/tampon insertion? Years, months:
- Did you have any period of normal functioning before your pain problem started? No Yes

HAVE YOU RECEIVED ANY OF THESE TREATMENTS

- Topical lidocaine No Yes
- Topical ointment No Yes
- Pelvic floor muscle rehabilitation No Yes
- EMG biofeedback No Yes
- Botox No Yes
- Amitriptylin No Yes
- Psycho-social counseling No Yes
- Surgery No Yes
- Any other treatment No Yes
  If yes: What treatment?

TREATMENT OUTCOME

- Did you complete your treatment at our clinic? No Yes
  If yes: do you think the treatment made your symptoms (Choose one of the five options)

  - Worse
  - No change
  - Improvement
  - Major improvement
  - Complete recovery

- Did you receive any other treatment after completing your treatment at our clinic? No Yes
  If yes: What treatment?

- How would you rate your symptoms today? (Chose one of the four options)

  - Never pain
  - Occasional mild pain that does not prevent vaginal intercourse
  - Moderate pain that sometimes prevent vaginal intercourse
  - Severe pain that makes vaginal intercourse impossible

- Please rate the intensity of coital pain during the last month by marking the line with an x!

No pain ----------Worst pain imaginable

- Please rate the level of your sexual desire during the last month by marking the line with an x!

No desire----------Maximal desire

- Do you currently have a sexual relationship? No Yes
  If yes: how long have you been in this relationship?

- If you have given birth how have the pain been since the delivery (chose one of the four options)

  - Worse
  - No change
  - Improvement
  - Complete recovery

OTHER COMMENTS

.....
.....

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