Genetics and Labor Pain Behavior

FATIMAH DABO PETTERSSON
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Abstract

Labor may perhaps be the most painful a woman might experience, although characterized by large inter-individual variability. The perceived pain during labor is the result of diverse factors, i.e. her previous pain experiences, the analgesia she receives and maybe also her genes. The overall aim of this thesis was to investigate biological and psychological mechanisms underlying inter-individual differences in labor pain related behaviors.

The mechanisms that characterize endogenous pain relief during labor are not fully understood, though it is known to be partly explained by the effects of β-endorphin (BE). BE plasma levels were followed longitudinally in a cohort of pregnant women and were found to remain unchanged between early and late pregnancy, although with a nadir in the beginning of the third trimester. Furthermore, women with low levels of BE in plasma at the end of the third trimester, required second line labor analgesia to a significantly higher extent than women with normal levels.

In a population-based sample of 814 pregnant women we investigated if inter-individual differences in labor pain related behavior was influenced by the pain-protective single nucleotide polymorphism (SNP) combination of guanosine triphosphate cyclohydrolase (GCH1) and the opioid receptor μ-1 gene (OPRM1) A118G SNP. We identified a possible association between the pain-protective SNP combination of GCH1 and use of second line analgesia. No association was found between the OPRM1 and use of analgesia or labor pain related behavior.

The association between self-rated antenatal depressed mood and anxiety in relation to pain behaviors and self-reported pain during labor was investigated. We found that depressed mood during pregnancy is associated with early arrival to the delivery department, whereas antenatal anxiety is associated with increased self-rated pain prior to labor analgesia.

In conclusion, although an increasing number of studies strongly suggest that genetic predisposition plays an important role in pain and pain-related mechanisms, GCH1 and OPRM1 has little to offer in terms of individual counseling on labor analgesia. To enable the future use of genetic variability for pre-labor testing and counseling, a number of different genes reflecting pain mediation pathways, involving biological and psychological mechanisms, need to be analyzed in combination.

Keywords: anxiety, beta-endorphin, depressed mood, GCH1, labor analgesia, labor pain, OPRM1, SNP

Fatimah Dabo Pettersson, Uppsala University, Department of Women’s and Children’s Health, Obstetrics and Gynaecology, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

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urn:nbn:se:uu:diva-162539 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-162539)
To my mother Filijay T Sawo, and her mother Binta Touray (Kunjur), and her mother Touréngding Jamba, and her mother Bintou Danso, and her mother Khadija “Banna” and her mother Mai Touray.
List of Papers

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


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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotrophic hormone</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BE</td>
<td>β-Endorphin</td>
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<tr>
<td>BH4</td>
<td>6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4)</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<tr>
<td>DNIC</td>
<td>Diffuse noxious inhibitory control</td>
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<tr>
<td>EDA</td>
<td>Epidural analgesia</td>
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<tr>
<td>GCH1</td>
<td>Guanosine triphosphate cyclohydrolase gene 1</td>
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<tr>
<td>GWA</td>
<td>Genome wide association</td>
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<tr>
<td>MADRS-S</td>
<td>Montgomery-Åsberg depression rating scale (self-rated version)</td>
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<tr>
<td>MC1R</td>
<td>Melanocortin-1 receptor gene</td>
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<tr>
<td>MOR</td>
<td>Human μ-opioid receptor</td>
</tr>
<tr>
<td>N₂O</td>
<td>Nitrous oxide</td>
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<tr>
<td>OPRM1</td>
<td>Opioid receptor μ 1 gene (human)</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
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<tr>
<td>PBI</td>
<td>Present behavioral intensity</td>
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<tr>
<td>PCB</td>
<td>Paracervical block</td>
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<tr>
<td>PDB</td>
<td>Pudendal nerve block</td>
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<tr>
<td>POMC</td>
<td>Pro-opio-melanocortin</td>
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<tr>
<td>SIA</td>
<td>Stress induced analgesia</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>STAI-T</td>
<td>Spielberger state-trait anxiety inventory (trait version)</td>
</tr>
<tr>
<td>TENS</td>
<td>Transcutaneous electrical nerve stimulation</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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Introduction

Labor pain

Labor is considered to be one of the most painful events in human experience and women often underestimate the pain they will experience (1). Many factors might influence the actual perception of pain i.e.; the size of the fetus, the duration of labor, cultural influences, previous painful experiences and the support the woman in labor receives. Endogenous pain modulators are also involved in the overall experience of labor pain, both in physiological and psychological response to the exposure of pain (2, 3).

The character of labor pain changes across the first and second stage of labor (4). In the first stage of labor the pain mainly emerges from mechanical distension of the lower uterine segment, mechanical dilation of the cervix and uterine-muscle contractions (5). Neuromechanically, the uterus and the cervix are supplied by afferent neurons accompanying the sympathetic nerves, the uterine and cervical plexus, the aortic plexus, the middle, superior and inferior hypogastric plexus. Small unmyelinated visceral C-fibers (slow transmitters) conduct nociception (6). Contraction pain is referred to the dermatomes supplied by T10, T11, T12 and L1 (5).

Labor pain in the second stage of labor is caused by mechanical distension of the lower uterine segment and cervix (similar to the first stage) but pressure from the presenting part of the fetus within the pelvis is also of importance. Neuropathic pain is thought to develop from contact pressure by the presenting part to the lumbosacral plexus (5). The second stage labor pain is characterized by a sharp, localized sensation in the perineal and recto-anal area, sometimes also in the lower extremities. A possible explanation to this sensation is traction and pressure in the urogenital organs, ligaments, muscles and fascia of the pelvic floor. This in turn stimulates the pudendal nerve originating from S2-S4. The stretching of vagina and perineum is transferred by myelinated rapidly transmitting A-delta fibers (6).
Physiological response to pain during labor

Physiological responses to labor pain include hyperventilation and metabolic changes with increased catabolic activity based on rising levels of glucagon and growth hormone, and decreases in anabolic hormones such as insulin and testosterone (7). Other hormonal changes that follow as a response to labor pain are increased release of cortisol, adrenocorticotropic hormone (ACTH) and β-endorphin (BE) (8, 9). Moreover, in response to maternal stress increased levels of noradrenaline and adrenaline have been found (9-11). The importance of plasma catecholamines and cortisol for maternal stress during labor has been proven since epidural or spinal analgesia result in decreased levels (12). Other evidence of augmented sympathetic activity includes a progressive rise in peripheral resistance and increased cardiac output (10, 11). Furthermore, increased levels of circulating free fatty acids and lactate during labor has been reported (13).

Labor pain and mental health

Emotional wellbeing and pain processing are intimately linked but has thus far most commonly been studied in the area of chronic pain. Longstanding and chronic pain has been shown to result in social isolation, depression and hopelessness (14, 15). Depression can render otherwise tolerable pain unbearable (16), and notably, antidepressants acting on the monoaminergic systems are commonly used to treat chronic pain (17).

Numerous psychological factors, however, have been associated with labor pain including expectations (18, 19), coping strategies (20), partner or doula support (21, 22), support from caregivers, and involvement in decision making throughout labor (23).

However, few studies have evaluated antenatal depression and anxiety as possible predictors of labor pain experience. It has been hypothesized that anxious women may fear that pain experiences *per se* are threatening and harmful thus influencing the labor pain experience (24). A number of studies have suggested that prenatal anxiety or anxiety sensitivity is associated with expected, experienced and recalled pain during labor (18, 24, 25). Women with high scores of anxiety appear to be more inclined to use labor analgesia during the second stage of labor (26), whereas women with high depression scores received overall more pain relief during labor (27). In addition, pregnant women with anxiety and/or depressive disorders appear to be more inclined to use epidural analgesia during labor (28). However, none of these studies adjusted for all factors that may influence use of labor analgesia.
Labor analgesia
There are a number of different techniques for labor analgesia available and in Sweden almost all women are offered similar techniques, independent of where they give birth. Regional differences are, however, found for frequency of use for some of the analgesics. The analgesic treatment during labor can briefly be categorized into the following groups; non pharmaceutical, inhalation, local anesthetic and neuraxial techniques.

Non-pharmaceutical techniques
The non pharmaceutical techniques are analgesic treatments that do not involve any pharmaceutical products and are thought to act through the patient’s endogenous pain relieving pathways. The non pharmaceutical methods used are transcutaneous electrical nerve stimulation (TENS), massage (29), water bath (30), intradermal sterile water injections, acupuncture and continuous support in labor.

Transcutaneous electrical nerve stimulation
TENS is a low electric current administered through electrodes attached to the skin. It acts on the posterior roots of the nerves supplying the uterus (31). The mechanism of nociceptive neurons contending each other is called “counter irritation” (32), which is explained by stimulation of nerves in the skin sharing the same dermatomal distribution as the uterus. It might offer some pain relief in the first stage of labor but is scarcely sufficient as the only analgesia used (33-36).

Intradermal sterile water injections
The intradermal sterile water injections entails that sterile water or saline is injected sub- or intracutaneously in the lower back and the sacrum. This offers the woman a stout but brief analgesia in the back or perineal area (37). The mechanism causing analgesia is thought to be based on pain inhibition from the periaqueductal grey (PAG) (32).

Acupuncture
Acupuncture is a technique for pain relief based on ancient Chinese medicine. The technique is to manually insert acupuncture needles into acupuncture points and applying manipulations; twisting, pulling and pulling of the needle until the patient and acupuncturist both experiences the “De Qi” sensation (38, 39). Acupuncture is thought to work through mechanisms of endorphin release due to hypothalamic activation and altered levels of neurotransmitters in the body (40). Acupuncture appears to be most effective in the first stage of labor (38, 39), but has a definite role in reducing pain (41) and may reduce the need of epidural analgesia (42).
Inhalation techniques

Some centuries ago different inhalation methods were common for labor analgesia, but today there is only one left in use, nitrous oxide (N₂O). N₂O is administered through a mask held over the nose and mouth and is co-distributed with 50 – 70 % oxygen. N₂O may be quite effective if the correct inhalation technique is used and if side effects as nausea and vomiting can be avoided. N₂O is often used when neuraxial methods cannot be offered or when progress in labor is rapid. It is used by approximately 81.5 % of women in labor (43).

Local anesthetic techniques

Paracervical nerve block

Paracervical nerve block (PCB) offers pain relief by blocking the sensory and sympathetic nerves in cervix (44). It is applied as an injection of local anesthetics into the paracervical tissue and it is mainly used during the first stage of labor. PCB has a relatively short duration and the injection can be repeated once. The use of PCB is 1.2 % in laboring women (43).

Pudendal nerve block

The pudendal nerve block (PDB) is used during the second stage of labor or after delivery when perineal injuries are sutured. Usage of PDB during the second stage of labor has decreased due to the increased use of EDA. PDB is used by 2.8% of women in labor (43).

Neuraxial anesthesia

A number of different neuraxial techniques are available; i.e. continuous epidural anesthesia (EDA), combined spinal-epidural analgesia, patient controlled epidural analgesia and spinal anesthesia (45).

Epidural anesthesia

EDA (Figure 1) is the most potent neuraxial anesthesia available. EDA acts through blocking the spinal segments of T11 and T12 and the sacral plexus (45). The woman in labor is commonly not offered EDA as the primary option for labor analgesia. It is given as the second or third alternative after the effect of N₂O has been evaluated.

It is the labor analgesia technique that provides the best pain relief and maternal satisfaction (46, 47). According to a relatively recent Cochrane review, epidural analgesia is associated with an increased risk of instrumental vaginal birth but not caesarean delivery, long-term backache or low neonatal Apgar scores at five minutes (46). However, in the Swedish context,
the introduction of bupivacaine and sufentanil EDA was associated with decreased rates of both instrumental vaginal deliveries and caesarean sections (48-50). EDA is used by 30.2% of all Swedish women in vaginal delivery (43), but the user frequency differs across the country, with most frequent use in Stockholm and middle-north of Sweden (51). Use of epidural analgesia in Sweden is associated with nulliparity, birth weight and a prenatal belief that epidural analgesia will be needed (51, 52).

![Epidural Analgesia Technique](image)

**Figure 1.** Epidural analgesia technique. Reproduced from Findley and Chamberlain, BMJ, 1999; 318: 927-930 with permission from BMJ Publishing Group Ltd.

### Measurement of pain

Pain is a highly subjective experience influenced by learning, context, attention, and other psychological variables. Various methods are used to measure pain including verbal and numeric self-rating scales, behavioral observation scales, and physiologic responses, but none of them detain all the aspects or dimensions of pain (53). Because pain is subjective, it has been argued that self-reported pain provides the most valid measure of the experience (53, 54).

In clinical practice perhaps the most commonly used approach is the visual analogue scale (VAS). It consists of a 100 mm line, with descriptions of the pain attached to it. The scale ranges between “no pain” (0 mm) to “worst imaginable pain” (100 mm). The patient is instructed to put a mark on the
100 mm line, corresponding to how she rates the pain (54-56). VAS is an ordinal scale, meaning that a score of 4.0 is not necessarily twice as painful as the score of 2.0. Usually the rating is done before or after an event i.e. pain relief. The major limitation with the VAS is that is has to be done on paper, a ruler or electronically (57), but it is simple and can be used and assessed promptly.

Other self-reported methods available to measure pain are the verbal rating scale and the McGill pain questionnaire. The verbal rating scale is also ordinal and includes adjectives to describe pain intensity; no pain, mild pain, moderate pain and severe pain (54, 58). The McGill pain questionnaire includes not only questions concerning pain but also queries about the socioeconomic situation. Moreover, the patient is asked to rank words describing the pain (59). Within the field of chronic pain the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials has recommended that additional core outcome domains should be assessed including physical and emotional functioning and patient satisfaction (60). Specific self-reported questionnaires for use in chronic pain have been developed but will not be addressed in this thesis.

Behavioral observation scales are most often used in patients who otherwise cannot express pain (patients with profound intellectual and severe motor disabilities, patients in anesthetic or intensive care, and neonatal infants) and include the Pain Behavior Checklist (61), the Iowa Pain Thermometer, Checklist of Nonverbal Pain Indicators (62), The Critical Care Pain Observation Tool (63), Pain Observation Scale for Young Children, Premature Infant Pain Profile, the COMFORT behavior scale (64) and many more. The Present Behavioral Intensity Scale (PBI) can be assessed by the attending midwife or obstetrician (65).

Physiologic responses to pain include blood pressure, heart rate, respiratory rate, and oxygen saturation. More recently, skin conductance has been introduced as an objective measure of pain in the neonatal (66) and post-operative units (67-70). The skin conductance level depends on the activity of the eccrine sweat glands (which reflects the output of the sympathetic nervous system) but has thus far only been sparsely used during labor (71). More advanced methods for establishing pain sensitivity and pain thresholds include, for instance, quantitative sensory testing with thermometry or pressure (72).

Endogenous pain modulators

The physiological mechanisms of endogenous anti-nociception have been subject to numerous studies but are still not fully understood. Endogenous pain modulation is in part explained by the endorphins, but numerous other substances are of importance as well. Among the most well-known sub-
stances are found endocannabinoids (73), norepinephrine (74), substance P (75), neuropeptide Y (76), serotonin (77), dopamine (78), N₂O (beyond ongoing use) (79) and oxytocin (80, 81).

The endorphins are anti-nociceptives that are released intra-cerebrally and spinally (80). Endorphins bind to the same receptors as and share similar analgesic effects with the opiates i.e. morphine, fentanyl and methadone (82). The endogenous opioids are of importance in regulating the organism’s response to physical and psychological stress. More than ten opioid peptides that derive from three different precursors of endorphin; pro-opiomelanocortin (POMC), pro-enkephalin and pro-dynorphin have been described (83). The opioid peptides bind with high affinity to the opioid receptors; μ-, δ- and κ-receptors.

β-endorphin

BE derives from POMC. The main production of BE takes place in the pituitary (anterior lobe) (84-86), the medial basal hypothalamus and nucleus commisuralis (87, 88). BE and ACTH are released simultaneously into the blood in response to stress and other ACTH-releasing stimuli. In peripheral tissue ACTH opposes the effects of BE and vice versa, but ACTH has in experimental studies, administered into brain tissue been shown to evoke analgesia (89).

BE binds to the μ- (also called the morphine receptor) and the δ-receptor, and in vitro it has a higher affinity for δ-receptors (89). The μ-receptor is found in the hypothalamic and thalamic nuclei, the PAG, and the median raphe nucleus. The δ-receptors are more prominent in the amygdala, nucleus accumbens, olfactory tubercle and the pontine nuclei (90).

β-endorphin in labor and pregnancy

It has been suggested that BE is of importance for pain modulation during pregnancy and labor (91). Furthermore, a study has indicated that women who exercise during pregnancy respond with increased BE levels and also report lower scores of pain during labor (92), whereas low BE levels were detected in patients with chronic stress and major depression (93, 94).

Few attempts have been made to relate BE levels at the end of pregnancy to clinical outcomes. A number of previous cross-sectional studies have assessed BE levels during pregnancy, (95-99) but only a few have included women longitudinally throughout pregnancy (100, 101). Both of the two latter longitudinal studies have reported gradual increases in BE plasma levels across pregnancy (100, 101), but sample size and time-frames for sampling were limited. The reason why BE levels increase during pregnancy (if they do) is not known, but it has been speculated that BE levels reflect increased stress at the end of a pregnancy or increased production from placental tissue (92, 102).
Furthermore, a pronounced elevation of BE has been described during labor and the early postpartum period (99, 103). High levels of BE during labor has been hypothesized to relate to increased use of analgesia, increased distress, and vaginal delivery (as opposed to caesarean section) (92, 104).

**Enkephalins and dynorphins**

Enkephalins are present in the anterior and posterior lobe of the pituitary, they are widely distributed in the central nervous system (i.e. the retina, vagal nerve and the brain), the adrenal medulla and the gastrointestinal tract (105-107). Six opioid peptides derive from pro-enkephalin; [Met]enkephalyl-Arg-Phe, [Met]enkephalyl-Arg-Gly-Leu, [Met]enkephalyl-Arg-Arg-Val- NH₂, leucine-enkephaline and methionine-enkephalin (83). They are present in the adrenals but in a smaller quantity also in the brain (82). The enkephalins have affinity for both μ- and δ-receptors (83).

Five opioid peptides arise from pro-dynorphin; α-neo-endorphin, β-neo-endorphin, dynorphin A, dynorphin B and dynorphin A (83). Dynorphins are mainly found in the anterior and posterior lobe of the pituitary, PAG and substantia nigra and analgesia is induced experimentally when injected into the spinal cord of rats (108). The dynorphins bind preferably to κ-receptors but unlike the other endorphins their effect is only partially reversible by naloxone (an opiate antagonist), indicating that their actions are not only mediated by opioid receptors (83).

**Anti-nociceptive mechanisms mediated by the endorphins**

Endogenous modulation of pain is often described by the central descending regulation from the PAG, but also as stress-induced analgesia (SIA), diffuse noxious inhibitory control (DNIC), and placebo response. Endorphins are thought to be involved in all of these mechanisms. Placebo response, SIA and DNIC are completely or in part reversible by naloxone, thus indicating the involvement of the opioid-receptors (109-111). BE is the most potent endorphin and believed to be an important element in PAG-induced analgesia, SIA, DNIC and placebo response.

Pain is modulated via a descending tract (Figure 2) from the midbrain in the PAG via the rostral ventral medulla down to the dorsal horn of the spinal cord (80). The PAG is also interconnected with the cerebral cortex and the amygdala (Figure 2), areas that are of importance for experience and fear of pain (112). Afferent nociceptive fibers from peripheral parts of the body are relayed in the spinal dorsal horn. Impulses from the afferent nociceptors are inhibited by axons from PAG at the spinal level. In this descending tract (top-down pain modulation) μ-receptors are thought to be part of the inhibitory influence on the afferent nociceptors in the spinal cord. The effects of non steroid anti-inflammatory drugs and opioids are thought to act through the top-down pain modulation (111, 113). The PAG-induced analgesia has
been reproduced experimentally through electric stimulation of the neurons in the PAG where an increased level of endorphins in the cerebrospinal fluid is subsequently seen (89).

SIA is the term for a strong analgesia evoked by endorphins that are released in response to a profound noxious stimulus. In order for SIA to be induced, the individual has to be under an extremely stressful condition and the pain stimulus has to exceed a certain threshold (80). The underlying mechanism is unknown but it can be reproduced in experimental pain studies and is thought to explain painlessness in certain conscious multi-trauma patients (89, 114).

DNIC corresponds to a peripheral noxious stimuli suppressing neuronal response to pain (115, 116). DNIC is believed to be inhibited in patients with chronic pain syndrome (117).
Figure 2. Top-down endogenous pain modulation. Reproduced from Ossipov et al, J Clin Invest. 2010; 120: 3779 – 3787, with permission of American Society for Clinical Investigation. LA-lateral amygdala, BLA - basolateral amygdala, CeA - central nucleus amygdala, PAG - periaqueductal gray, LC - locus coeruleus, RVM - rostral ventromedial medulla, DRt - dorsal reticular nucleus
Single nucleotide polymorphism

Besides the role of endogenous pain modulation, a number of recent studies strongly suggest that genetic factors play an important role in the mechanisms underlying the experience of pain (118-120).

Single-nucleotide polymorphisms (SNPs) are single base-pair alterations in the deoxyribonucleic acid (DNA) sequence and represent a major source of genetic heterogeneity. This heterogeneity will influence how people differ in their risk of disease or their response to drugs. About 10 million SNPs exist in human populations, where the rarer SNP allele has a frequency of <5% (121).

In general, SNPs occur less often in coding regions of the genome than in non-coding regions (122, 123). SNPs located in the regulatory sites of a gene can affect the rate of transcription, ultimately affecting the production of the specific encoded protein. When present in the coding regions, SNPs can cause alterations in protein structure and hence its function (124). The genetic variance due to a SNP may vary between different ethnic groups, because of population stratification, i.e. Africans and ethnic groups with proximity to Africa have more SNPs than some populations in North America, Asia and Europe (121).

An allele corresponds to a viable part of the DNA sequence located at a given locus on the chromosome. A set of associated SNP alleles in a region of a chromosome is called a haplotype. These closely linked genes are usually inherited as a unit. The international HapMap project (http:// hapmap.ncbi.nlm.nih.gov/whatishapmap.html.en) "tag" SNPs that uniquely identify these haplotypes are monitored with the purpose of identifying regions in the chromosomes that associate with specific diseases or behaviors.

SNP and pain sensitivity in humans

Besides one genome-wide association (GWA) study in post-surgical pain (118), most interest has been devoted to candidate gene association studies. These candidate genes mainly fall into two categories; neurotransmission modulators and mechanisms that affect inflammation (119). Some of the most studied genes include the guanosine triphosphate cyclohydrolase 1 (GCH1) gene, the human opioid receptor μ-1 (OPRM1) gene, the melano-cortin-1 receptor gene (MC1R) and the catechol-O-methyltransferase (COMT) (125).

The opioid receptor μ-1 gene

The OPRM1 gene encodes for the human μ-opioid receptor (MOR). The MOR is of importance in pain modulation, through stimuli from both endogenous and exogenous substances. As the main receptor for BE, it is of im-
portance for the physiological and psychological response to stress, trauma and pain. The MOR also serves as a main receptor for opiates, in clinical practice morphine and other opiates, which are widely used for pain relief. Due to large individual differences in doses needed for pain relief and tolerance of opiates, there is a need for increased knowledge about the mechanisms regulating that.

A number of SNPs in OPRM1 has been hypothesized to be of importance for inter-individual differences in morphine-induced analgesia and pain sensitivity (126, 127). The OPRM1 A118G SNP has been shown to be of main relevance and in this specific polymorphism an adenine nucleotide is replaced by guanine, which results in a change of asparagine to aspartate at position 40 in the µ-opioid receptor protein and a putative glycosylation site at the N-terminal part is eliminated (128). The gene is located on chromosome 6, contains 4 exons and is 236371 base pairs (http://genome.ucsc.edu/). In vitro studies of the OPRM1 A118G SNP have indicated a three-fold increase in affinity and potency of BE on homozygous G allelic receptors (128). It has thus, been hypothesized that wild type OPRM1 carriers need more analgesia for pain relief and have lower pain thresholds and tolerance than the G-allele carriers (homozygous and heterozygous for A/G). In clinical studies, the specific polymorphism affects both subjective pain reporting and objective pain-related cortical activity (129). Opioid analgesic needs in chronic pain could also be affected by this polymorphism (130) and the requirement of intrathecal fentanyl analgesia in laboring women is influenced where the 118G allele carriers used lower levels of fentanyl suggesting that they are more responsive to opioids than wild-type A118 allele carriers (131). On the contrary, Wong et al could though not identify any association between duration of analgesia and treatment for breakthrough pain based on intrathecal opioid analgesia during labor and the A118G polymorphism of OPRM1 (132). Furthermore, individuals homozygous for the wild-type A118 allele required less morphine to manage early pain after total abdominal hysterectomy (133). Hence, the role of this polymorphism in influencing pain perception and analgesic requirement is controversial and not fully elucidated.

**Guanosine triphosphate cyclohydrolase 1**

It has been reported by Tegeder and colleagues that the pain-protective SNP combination of guanosine triphosphate cyclohydrolase gene 1 (GCH1) is associated with reduced pain sensitivity in humans (134). Guanosine triphosphate cyclohydrolase 1 is the rate limiting enzyme in the biosynthesis of 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) (135, 136). BH4, in turn, is an essential cofactor in the synthesis of many pain modulators including catecholamines, serotonin and N20 (137) and feed-forward activation via phenylalanine and feedback inhibition through BH4 regulates the activity of GCH1 (138). The identified pain-protective SNP combination of GCH1 is
composed of 15 SNPs found at different locations on the gene (134). Recently, screening for the combination of three SNPs has been shown to be sufficient for definition of the pain-protective haplotype with high sensitivity and specificity: c.-9610G > A, c.343+8900A > T, and c.*4279 > G (139). The literature on the pain-protective SNP combination of GCH1 is conflicting and the pain-protective effect appears to be most evident in patients with neuropathic rather than nociceptive pain. Tegeder and colleagues (134) suggested that the pain-protective SNP combination of GCH1 is associated with less pain following discectomy for persistent radicular low back pain and their results have later been reproduced both by themselves (140) and by others (141). Further studies on the pain-protective SNP combination of GCH1 have been more discouraging.
Aims

I. to measure how plasma levels of BE vary during normal pregnancy and to investigate whether plasma levels of BE in late pregnancy are associated with a number of pain related behaviors during labor.

II. to investigate if there is a clinically relevant association between the pain-protective SNP combination in the guanosine triphosphate cyclohydrolase 1 (GCH1) gene and a number of pain related behaviors during labor.

III. to investigate whether there is a clinically relevant association between the A118G polymorphism of the human opioid receptor μ-1 gene (OPRM1) and a number of pain related behaviors during labor.

IV. to assess if self-rated antenatal depressed mood and anxiety are associated with pain related behaviors and self-reported pain during labor.
Material and Methods

Study populations

Study I

Study I was based on a random sample from a cohort of healthy pregnant women (n = 469) with singleton pregnancies enrolled in gestational weeks 8 to 12, at five antenatal healthcare centers in Värmland County, Sweden. The women were followed longitudinally during pregnancy and plasma samples were collected at scheduled routine visits at the antenatal health care centers at gestational weeks 10, 25, 28, 33 and 37. The random sample consisted of women (n=45) with at least 4 blood samples (and at least 1 blood sample from gestational week 37) throughout pregnancy. Exclusion criteria were pregnancy complications such as hypertension or preeclampsia, known malformation of the fetus, premature labor, late onset of labor (after week 42 + 0), planned or acute caesarean section and induced start of labor or chronic diseases. In addition, women with subjective reports of pelvic pain in early pregnancy, daily use of analgesics, or drug abuse were excluded.

Study II-III

Between March 1, 2007 and May 31, 2007 all women (age > 18 years) attending the 2nd trimester routine ultrasound screening at Uppsala University Hospital were approached for study participation. In Uppsala County, all routine ultrasound examinations are performed at Uppsala University Hospital which also is the only available delivery ward within the county. Hence, the study subjects represent a population-based sample. Among all eligible women, 814 (approximately 65% of eligible pregnant women during the inclusion period) accepted to participate in the study. Exclusion criteria at this point of the study were 1) detection of malformation or missed abortion at the ultrasound examination, 2) inability to read and understand the study information due to language difficulties, and 3) not providing informed consent.

Following the review of the medical records additional exclusion criteria were applied: Women delivering elsewhere, with intrauterine fetal death or gestational length < 30 weeks (< 33 weeks in paper III), planned or emergency caesarean section before onset of labor and non-Caucasian origin. The patient population of paper II-III is described in figure 3.
Figure 3. Flow-chart of the patient population of papers II and III. Numbers in parentheses refer to paper III where women delivering prior to gestational week 33 were excluded. One non-Caucasian women delivered prior to gestational week 33, thus reducing the number of non-Caucasians left to exclude in the flow-chart.

Study IV
In the fourth and final study, 99 women in gestational weeks 37 to 40 were recruited through public maternity health care units in Uppsala County and through local newspaper advertisement. Healthy primiparous and multiparous women above 18 years of age with an uncomplicated singleton pregnancy were eligible for inclusion. The exclusion criteria were severe pregnancy complications (pre-eclampsia, intrauterine growth retardation) and planned caesarean section.

In all studies demographic data and information on analgesia use during labor, together with other obstetric and delivery variables, were retrieved from the medical records of the parturients. Each participating woman gave her written informed consent and the studies were approved by the Independent Ethical Review Board at Uppsala University, Sweden.
Methods

Sample collection
In study I blood samples were collected in Lithium/Heparin containing tubes and centrifuged for 10 min at 3000 rpm. Plasma was transferred into tubes and stored at -70°C. In study II-IV blood samples were collected in tubes containing EDTA and the samples were centrifuged for 10 minutes at 5000 rpm. Plasma and buffy coat were separated and stored in new tubes at -70°C.

Measurement of β-endorphin in plasma
To analyze levels of BE in plasma the radioimmunoassay (RIA) EURIA-beta-Endorphin kit (Euro-Diagnostica AB, Sweden) was used. This RIA is based on double-antibody precipitation. No levels were below the detection limits at any time point during pregnancy. All samples from each woman were run in duplicate and in the same assay to reduce variability. No levels were below the detection limit (10 pg/mL) at any time point during pregnancy.

DNA isolation study II-IV
Total genomic DNA was extracted from whole blood using the Magtration 12GC system (Precision System Science, Chiba, Japan) and the Magazorb® DNA Common Kit-200 (Precision System Science, Chiba, Japan) as described earlier (142).

SNP analysis
The different SNPs were determined by the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). Applied Biosystems designed the target specific PCR primers and allele-specific TaqMan MGB probes (assay numbers GCH-1; C_25800745, C_1545138 and C_3044867, respectively, and OPRM-1; C 8950074 1) labeled with FAM and VIC. The assays were performed according to the manufacturer’s instructions.

Administration of labor analgesia
Labor analgesia (N2O, TENS, acupuncture, sterile water blocks, PCB, PDB and EDA) was administered according to the routines at the delivery ward. All types of analgesia were at available for the women in labor at all times. Details concerning the different kinds of analgesia used are presented in the manuscripts.

Labor Pain-Related Outcomes
The primary labor pain-related behavior outcomes for the entire thesis were 1) stage of cervical dilation on arrival at the delivery unit and 2) use of any type of second line analgesia. Second line labor analgesia was defined as any use of more than one form of analgesia during labor. Following review of
paper I, it was specifically asked that we should use a slightly different outcome instead of second line analgesia i.e. need for additional pain medication beyond N₂O during labor (the change of outcome measure did not alter the results). Additional pain-related outcomes have been added in paper II and III, based on the request of the reviewers.

**Visual Analogue Scores**

In study II-IV, each woman was asked to retrospectively rate labor pain (within 24 hours of delivery). Due to organizational difficulties and as women were recruited while still pregnant, it was considered unfeasible to perform prospective pain ratings during labor. On two visual analogue scales women rated labor pain prior to labor analgesia and during the last two hours of labor, irrespective of analgesia use. The scale measured from 0 to 100 mm where 0 equaled no pain and 100 represented worst possible pain. However, VAS scores were only returned by 337 of 676 women in study II –III (response rate 49.8 %), and for this reason, were not included in papers II – III. The results of the VAS scores have been added to the thesis as they provide valuable information of the outcome measures that were used and to the difficulties of measuring pain in the labor ward.

**MADRS-S and STAI-T**

Women who participated in paper IV filled out the self-rated version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S) (143) and the trait version of the Spielberger State-Trait Anxiety Inventory (STAI-T) (144) 14 days prior to expected delivery. Depressed mood was defined as a score ≥11 on the MADRS-S scale (143). A STAI-T score > 40 was used to indicate increased anxiety levels (144, 145). Both of these scales are commonly used in studies of pregnant women, and are available in validated Swedish versions (the MADRS-S was originally made in Sweden).

**Statistics**

**Study I**

Comparisons of BE plasma levels between different gestational weeks were made with repeated measures analysis of variance (ANOVA). Two different ANOVAs were made, one including all samples throughout pregnancy and the other including the third trimester samples. Multivariate logistic regression analysis was used to calculate adjusted odds ratios (AOR) for need for additional pain medication beyond N₂O during labor, with third trimester BE levels, together with a number of confounding variables, as independent variables.
**Study II-III**

Demographic and obstetric variables were compared between homozygous, heterozygous and non-carriers of the pain-protective SNP combination of GCH1 by one-way ANOVA and Tukey HSD or Chi-square tests. Any significant differences between groups were adjusted for relevant confounders by analysis of covariance (ANCOVA) or multivariate logistic regression. Demographic and obstetric variables were compared between non-carriers and G-allele carriers of the OPRM1 A118G SNP using Student’s T-test and Chi-square tests.

Multivariate linear (cervical dilation at arrival to the delivery unit) and logistic (use of second line analgesia) regression models were composed to address the influence of the different SNPs.

**Study IV**

MADRS-S and STAI-T scores were analyzed by Mann-Whitney U-test, as assumption of normal distribution was not met. Multivariate linear (cervical dilation at arrival to the delivery unit) and logistic (use of second line analgesia) regression models were composed.

The statistical analyses are described in further detail in each paper. All statistical analyses were performed by the Statistical Package for the Social Sciences (SPSS) 18.0 for Windows software package.
Results

Study I

Levels of BE during pregnancy decreased significantly in gestational weeks 28 and 33 compared to gestational week 10 (14.4 and 14.4 pg/mL compared to 16.6 pg/mL, p< 0.001 and p < 0.01, respectively, Figure 4). A significant increase in BE level between week 28 and 37 was noted, p < .01. However, there was no difference in plasma concentrations of BE between gestational weeks 10 and 37.

Stepwise multivariate logistic regression of the results with use of labor analgesia other than N₂O as the outcome variable and BE levels and possible confounders as predictor variables were performed. The

Figure 4. Mean ± SD levels of BE at different gestational weeks among women with complete blood sampling (n = 38). *** Significant decrease in gestational week 28 compared to gestational week 10, p<0.001. **Significant decrease in gestational week 33 compared to gestational week 10, p<0.001. # Significant increase in BE levels between gestational week 28 and week 37, <0.01.
Table 1. Factors associated with need for additional pain medication beyond N₂O during labor

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted OR (95 % CI)</th>
<th>Adjusted OR (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Primipara</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Multipara</td>
<td>0.20 (0.06 – 0.72)</td>
<td>0.39 (0.06 – 2.52)</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.00 (0.88 – 1.14)</td>
<td>1</td>
</tr>
<tr>
<td>Pre-pregnancy BMI &lt; 25.0 kg/m²</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 25.0 kg/m²</td>
<td>4.64 (1.24 – 17.4)</td>
<td>3.88 (0.54 – 28.0)</td>
</tr>
<tr>
<td>Dilation of cervix at arrival to labor ward, cm</td>
<td>0.62 (0.44 – 0.89)</td>
<td>0.53 (0.31 – 0.91)</td>
</tr>
<tr>
<td>Duration of labor, hours</td>
<td>1.92 (1.28 – 2.89)</td>
<td>1</td>
</tr>
<tr>
<td>Use of oxytocin during delivery</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>1.49 (1.01 – 2.07)</td>
<td>1.44 (0.82 – 2.50)</td>
</tr>
<tr>
<td>Gestational week, weeks</td>
<td>1.50 (0.80 – 2.82)</td>
<td>1</td>
</tr>
<tr>
<td>Smoking</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>0.79 (0.19 – 3.31)</td>
<td>0.75 (0.19 – 3.31)</td>
</tr>
<tr>
<td>Low BE levels ¹</td>
<td>11.5 (2.12 – 62.3)</td>
<td>18.67 (1.53 – 227)</td>
</tr>
<tr>
<td>Reference</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LQ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Subjects in the lowest quartile (LQ) of BE levels compared to the other quartiles at gestational week 37.

Marital status, ethnicity, labor onset, fetal presentation, delivery were not considered in the model because of the homogenous study group. Duration of labor was not entered in the final model as it correlated with cervical dilation at arrival to the labor ward. However, the odds ratio for low BE levels was not significantly altered by entering or not entering duration of labor to the final model.

Multivariable logistic regression analysis indicated that women within the lowest quartile of BE levels at gestational week 37 required labor analgesia other than N₂O to a significantly higher extent, and this finding remained after adjustment for possible confounders such as parity, duration of labor, cervical opening upon arrival at the delivery unit and use of oxytocin during labor (Table 1).

Study II

Among the 676 Caucasian subjects who had complete data on the SNP analyses in the study population, 15 (2.2%) were homozygous carriers, 180 (26.6%) heterozygous carriers and 481 (71.1%) were non-carriers of the pain-protective SNP combination of GCH1. Homozygous carriers, heterozygous carriers and non-carriers of the 3 SNPs used were in accord with the Hardy-Weinberg equilibrium. There were no major differences in sociodemographic and clinical variables between groups.
Table 2. Labor pain related behavioral outcomes according to GCH1 SNP combinations

<table>
<thead>
<tr>
<th></th>
<th>Non-carriers (n = 481)</th>
<th>Heterozygous (n = 180)</th>
<th>Homozygous (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical dilation at arrival to the delivery unit, cm</td>
<td>4.3 ± 2.5</td>
<td>4.1 ± 2.3</td>
<td>5.7 ± 2.2*</td>
</tr>
<tr>
<td>Cervical dilation at request of epidural analgesia, cm</td>
<td>5.8 ± 1.9</td>
<td>5.3 ± 2.0</td>
<td>6.3 ± 2.3</td>
</tr>
<tr>
<td>Use of labor analgesia, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No use</td>
<td>44 (9.1 %)</td>
<td>17 (9.4 %)</td>
<td>1 (6.7 %)</td>
</tr>
<tr>
<td>N2O</td>
<td>396 (82.3 %)</td>
<td>143 (79.4 %)</td>
<td>12 (80.0 %)</td>
</tr>
<tr>
<td>Acupuncture</td>
<td>155 (32.2 %)</td>
<td>62 (34.4 %)</td>
<td>6 (40.0 %)</td>
</tr>
<tr>
<td>Epidural analgesia</td>
<td>159 (33.1 %)</td>
<td>52 (28.9 %)</td>
<td>7 (46.7 %)</td>
</tr>
<tr>
<td>Second line analgesia</td>
<td>249 (51.8 %)</td>
<td>92 (51.1 %)</td>
<td>11 (73.3 %)</td>
</tr>
</tbody>
</table>

*p = 0.033 in comparison to heterozygous carriers and non-carriers of the GCH1 SNP combination, ANOVA adjusted for parity. a Data missing in 81 subjects. b Data based on 159 non-carriers, 52 heterozygous and 7 homozygous carriers of the GCH1 SNP combination.

Homozygous carriers of the pain-protective SNP combination of GCH1 arrived in the delivery ward with a more advanced stage of cervical dilation than subjects heterozygous for the pain-protective SNP combination of GCH1 and non-carriers did, adjusted for parity (Table 2).

A possible association between the pain-protective SNP combination of GCH1 and use of second line analgesia was suggested by the bivariate analysis (p = 0.10). This possible association was further analyzed with adjustment for confounders in a multivariate logistic regression model. In the bivariate analyses nulliparity, induced labor, cervical dilation upon arrival in the delivery unit, and duration of labor were associated with second line labor analgesia. Independent explanatory factors for use of second line labor analgesia use were nulliparity, dilation of cervix < 2 cm at arrival to the delivery unit and duration of labor for more than 2 hours.

Multivariate logistic regression indicated that homozygous carriers of the pain-protective SNP combination of GCH1 had an increased risk of using second line labor analgesia (OR 5.1, 95% CI, 1.09-23.96), whereas heterozygous carriers did not differ in this aspect from non-carriers (Table 3).
<table>
<thead>
<tr>
<th>Parity</th>
<th>Use of second line labor analgesia</th>
<th>Unadjusted OR</th>
<th>95 % CI</th>
<th>Adjusted OR</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parous</td>
<td>130 (34.9 %)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>222 (73.3 %)</td>
<td>5.12***</td>
<td>3.67 – 7.14</td>
<td>2.68***</td>
<td>1.73 – 4.16</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>0.98</td>
<td>0.96 – 1.00</td>
<td>0.98</td>
<td>0.96 – 1.00</td>
</tr>
<tr>
<td>Start of labor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>291 (50.2 %)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Induced</td>
<td>61 (63.5 %)</td>
<td>1.73*</td>
<td>1.11 – 2.70</td>
<td>0.63</td>
<td>0.32 – 1.23</td>
</tr>
<tr>
<td>Cervical dilation at arrival to the delivery unit, cm&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5</td>
<td>83 (36.9 %)</td>
<td>1</td>
<td>1</td>
<td>0.79 – 0.97</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>178 (56.9 %)</td>
<td>2.26***</td>
<td>1.59 – 3.20</td>
<td>1.34</td>
<td>0.85 – 2.13</td>
</tr>
<tr>
<td>0-1</td>
<td>45 (78.9 %)</td>
<td>6.42***</td>
<td>3.21 – 12.82</td>
<td>2.46*</td>
<td>1.05 – 5.76</td>
</tr>
<tr>
<td>Duration of labor&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2 hours</td>
<td>32 (17.6 %)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2-5 hours</td>
<td>74 (45.1 %)</td>
<td>3.85***</td>
<td>2.36 – 6.29</td>
<td>3.33***</td>
<td>1.92 – 5.80</td>
</tr>
<tr>
<td>5-10 hours</td>
<td>109 (66.1 %)</td>
<td>9.12***</td>
<td>5.54 – 15.03</td>
<td>7.13***</td>
<td>3.87 – 13.13</td>
</tr>
<tr>
<td>&gt; 10 hours</td>
<td>134 (87.0 %)</td>
<td>31.41***</td>
<td>17.14 – 57.53</td>
<td>16.53***</td>
<td>7.68 – 35.62</td>
</tr>
<tr>
<td>GCH1 pain protecting genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>249 (51.8 %)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>92 (51.1 %)</td>
<td>0.97</td>
<td>0.69 – 137</td>
<td>1.25</td>
<td>0.80 – 1.97</td>
</tr>
<tr>
<td>Homozygous</td>
<td>11 (73.3 %)</td>
<td>2.56</td>
<td>0.80 – 8.16</td>
<td>5.11*</td>
<td>1.09 – 23.96</td>
</tr>
</tbody>
</table>

<sup>a</sup> Missing cases 81, <sup>b</sup> Missing cases 11
Study III

Genotyping was successful in 672 of 675 included cases. Of these, 541 (80.5%) were homozygous carriers of AA, 119 (17.7%) were heterozygous carriers (AG) and 12 (1.8%) were homozygous carriers of GG. The number of homozygous carriers, heterozygous carriers and non-carriers of the OPRM1 A118G SNP were in accord with the Hardy-Weinberg equilibrium. All women included, were assigned to two groups according to genotype. Non-carriers (n = 541) corresponded to homozygous carriers of the wild-type allele (AA), and the G-allele group (n = 131) were heterozygous and homozygous carriers of the 118G allele.

No significant differences in socio-demographic variables or obstetric outcomes were found between non-carriers and G-allele carriers. However, induced labor tended to be more common in G-allele carriers, why subsequent analyses on pain-related outcomes only have included subjects with spontaneous start of labor.

Labor pain-related outcomes for non-carriers and G-allele carriers with spontaneous start of labor are shown in Table 4 and 5. Following adjustment for parity, no differences in cervical dilation on arrival at the delivery unit or cervical dilation at request of epidural analgesia were detected.

A multivariate logistic regression model was performed and odds ratios adjusted for parity, height and duration of labor were calculated. As seen in Table 5, no association between G-allele carriers and use of N₂O, acupuncture, epidural, morphine, second or third line analgesia was identified.

Table 4. Labor pain behavior related outcomes and the OPRM1 A118G SNP of in the 577 women with spontaneous onset of labor

<table>
<thead>
<tr>
<th></th>
<th>Non-carriers</th>
<th>G-allele carriers</th>
<th>β</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical dilation at arrival to the delivery unit, cm</td>
<td>4.5 ± 2.4</td>
<td>4.7 ± 2.6</td>
<td>0.02</td>
<td>0.62</td>
</tr>
<tr>
<td>Cervical dilation at request of epidural analgesia, cm</td>
<td>5.9 ± 2.0</td>
<td>5.9 ± 1.3</td>
<td>-0.02</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Linear regression, adjusted for parity
Table 5. Use of labor analgesia and the OPRM1 A118G SNP

<table>
<thead>
<tr>
<th></th>
<th>Non-carriers (n=471)</th>
<th>G-allele carriers (n=106)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No use</td>
<td>46 (9.8 %)</td>
<td>10 (9.4%)</td>
<td>1.23 (0.55 – 2.78 )</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>391 (83.0 %)</td>
<td>85 (80.2 %)</td>
<td>0.84 (0.48 – 1.49)</td>
</tr>
<tr>
<td>Acupuncture</td>
<td>149 (31.6 %)</td>
<td>33 (31.1 %)</td>
<td>1.04 (0.64 – 1.69)</td>
</tr>
<tr>
<td>Epidural analgesia</td>
<td>147 (30.2 %)</td>
<td>33 (31.1 %)</td>
<td>1.23 (0.69 – 2.20)</td>
</tr>
<tr>
<td>Second line analgesia</td>
<td>218 (46.3 %)</td>
<td>51 (48.1 %)</td>
<td>1.31 (0.77 – 2.24)</td>
</tr>
<tr>
<td>Third line analgesia</td>
<td>61 (13.0%)</td>
<td>12 (11.3%)</td>
<td>0.99 (0.48 – 2.05)</td>
</tr>
</tbody>
</table>

OR; Odds ratio (adjusted for parity, height and duration of labor), n.c. not calculated

Addendum to study II and III

Median VAS ratings prior to labor analgesia and during the last two hours of labor were 74 mm (0 – 100 mm) and 81 mm (2 – 100 mm), respectively. Both scores, but especially the VAS scores during the last two hours of labor, were highly skewed towards the higher end of the scale. Self-rated pain prior to labor analgesia was significantly higher among women who subsequently used second-line analgesia (first line analgesia users median 71 mm (0 – 100 mm) vs. second line analgesia users median 77 mm (16 – 100 mm), p < 0.05), but there was no difference between these groups during the last two hours (median 80 (9 – 100 mm) vs. 83 (2 – 100 mm), respectively). The latter finding, however, was significantly influenced by the use of epidural analgesia during the final stage of labor, Table 6.

No differences in self-rated pain between carriers or non-carriers of the pain-protective SNP combination of GCH1 or 118G allele of OPRM1 were found.

Table 6. Retrospectively self-reported pain according to use of labor analgesia, median (range)

<table>
<thead>
<tr>
<th></th>
<th>None or first-line analgesia only</th>
<th>Second-line analgesia other than epidural analgesia</th>
<th>Epidural analgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain during last two hours of labor, mm</td>
<td>n = 177</td>
<td>n = 65</td>
<td>n = 100</td>
</tr>
<tr>
<td>80 (9 – 100)</td>
<td>90 (31 – 100)**</td>
<td>80 (2 – 100)</td>
<td></td>
</tr>
</tbody>
</table>
*** p < 0.001 in comparison with none or first-line analgesia users and epidural analgesia users, Kruskal-Wallis followed by Mann-Whitney U-test.

Study IV

Of the 99 women included in the paper IV, one subsequently underwent a planned caesarean section and one woman was lost to follow-up as she delivered elsewhere. Among included women, 83 (85.6 %) had a spontaneous onset of labor and were evaluated in the analysis on cervical dilation at arrival to the delivery department, whereas the entire study group were included in the aims on second line analgesia. Sixteen (16.5 %) subjects had MADRS-S scores ≥ 11, indicating depressed mood but none scored above 20 which would indicate possible major depression. Only ten (10.3 %) subjects had STAI-T scores > 40, indicating increased anxiety.

Explanatory factors for stage of cervical dilation at arrival to the delivery unit are displayed in Table 7. Following adjustment for BMI and parity the MADRS-S score was significantly associated with cervical dilation at arrival to the delivery department.

Women who used second line analgesia reported higher scores on the MADRS-S (7.1 ± 4.1 vs. 5.4 ± 4.1 in none or first line users, p < 0.05) but did not differ in anxiety ratings. However, when adjusted for parity, BMI, gestational length, duration of labor and the pain-protective SNP combination of GCH1, the association between MADRS-S score and use of second line analgesia was lost, Table 8. Together with body mass index and duration of labor, GCH1 remained a significant explanatory variable for use of second line analgesia.

No differences in visual analogue ratings of pain were found between women with depressed mood (MADRS-S ≥ 11) and women with MADRS-S scores in the normal range. Women with high anxiety levels (STAI-T > 40) reported increased self-rated pain prior to labor analgesia in comparison with women who had anxiety scores in the normal range (88 mm (61 – 100 mm) vs. 72 (11 – 100 mm) in non-anxious women).
Table 7. Multivariate linear regression analysis on factors associated with cervical opening at arrival to the delivery department in 83 women with spontaneous onset of labor

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Parous subjects</td>
<td>0.213</td>
<td>0.053</td>
<td>0.196</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.234</td>
<td>0.034</td>
<td>-0.165</td>
<td>0.14</td>
</tr>
<tr>
<td>MADRS-S scores</td>
<td></td>
<td></td>
<td>-0.254</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Model 1: $R^2 = 0.08$, Model 2 $R^2 = 0.14$

Table 8. Multivariate logistic regression of variables increasing the risk of using second line analgesia ($n = 97$)

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95 % CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparity</td>
<td>1.47</td>
<td>0.44 – 4.92</td>
<td>ns</td>
</tr>
<tr>
<td>BMI</td>
<td>1.17</td>
<td>1.01 – 1.36</td>
<td>0.05</td>
</tr>
<tr>
<td>Gestational week at labor</td>
<td>0.99</td>
<td>0.65 - 1.54</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 hours</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5 hours</td>
<td>2.82</td>
<td>1.56 – 5.10</td>
<td>0.001</td>
</tr>
<tr>
<td>MADRS-S (total)</td>
<td>1.05</td>
<td>0.94 - 1.19</td>
<td>ns</td>
</tr>
<tr>
<td>pain-protective SNP combination of GCH1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-carriers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous/homozygous</td>
<td>3.51</td>
<td>1.18 – 10.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Nagelkerke $R^2 = 0.38$, CI confidence interval
Discussion

Methodological considerations
Safe pain relief with the highest maternal satisfaction for each parturient is central in obstetric care. Labor analgesia is nowadays, in our country, available for all women at the delivery units, but not all women are satisfied and do suffer from labor pain. To enable individual counseling on labor analgesia the possibility to identify candidate genes or genetic variants that regulate endogenous pain mechanisms would be of importance. In our work we have investigated a number of labor pain related outcomes and their association with endogenous pain modulation, different genes and SNPs of interest.

Labor pain-related behavior
Pain is a highly subjective experience. Various methods can be used to measure pain, but no single measurement will cover all aspects of pain (53). For this thesis, two primary pain-related endpoints were used, namely stage of cervical dilation on arrival at the delivery unit and use of any type of second line analgesia. These outcomes were chosen as information was readily available in the majority of the medical records. Vaginal palpation for assessment of cervical dilation, effacement and fetal station is performed at the arrival to the labor ward or before induction of labor. It is furthermore often done when epidural analgesia is requested. Amount and type of labor analgesia used is documented in the medical records and our definition of second line analgesia was any use of more than one type of analgesia during labor. By using this definition a larger cohort than women who ask for EDA was included in the analysis. However, these measures are confounded by a number of variables, some of which may not have been accounted for in this work.

Cervical stage at arrival to the delivery department was in our studies affected by parity, depressed mood, and (in one of the study populations also by BMI in the bivariate analysis). Besides the fact that data on depressed mood was not available for subjects in paper II and III, residual confounding may have included distance from the hospital, family support, and previous experience of rapid labor or history of obstetric complications. The usefulness of cervical stage as a measure of pain is, however, supported by a prior study where prospective pain ratings on the short form of the McGill pain
questionnaire were used (4). According to the findings of Capogna and colleagues, mean intensity of pain increased as cervical dilation increased in both nulliparous and multiparous women.

Second line analgesia was influenced by parity, height (BMI in paper IV), labor, cervical stage at arrival to the delivery department, duration of labor, onset of and use of oxytocin. The latter four variables are confounded and in paper III and IV, only duration of labor was used in the final analysis. The usefulness of second line analgesia as a measure of pain is strengthened by the visual analogue scores, where women in need of second line analgesia rated more severe pain prior to labor analgesia than women who used no or only first line analgesia. Clearly, the studies would have been improved if prospective and subjective measures of pain, at defined stages of labor (on admittance, before first line analgesia, before second line analgesia and so forth) had been included. In addition, behavioral observation scales, such as the PBI can be assessed by the attending mid-wife (65).

All women in this thesis were included while still pregnant. This procedure precluded the use of the visual analogue ratings in paper II and III, as more than half of women forgot to bring their pain-scales to the delivery unit when labor started. Clearly, in paper IV, where women were included much later in pregnancy, the visual analogue scores were remembered and filled out by the majority of subjects. Although the usefulness of the visual analogue scales were hampered, the inclusion of subjects already during pregnancy may have its’ advantages. Had women been included at admittance to the delivery unit, informed consent would have been taken while women were in pain, which may not be ethical. In addition, inclusion upon admittance would presumably target women with less behavioral pain.

Arbitrary power analyses were conducted prior to each of the studies. However, in the case of paper II, the sample size nevertheless became insufficient as the homozygous carriers of the pain protective SNP combination of GCH1 were few. The study population of paper II and III is also far more heterogeneous (in terms of the labor variables) than the other two study populations, and in retrospect, it would have been wise to apply more strict inclusion criteria for the study such as nulliparity and planned vaginal delivery.

β-endorphin and labor

Although a large part of the research regarding BE has decreased in recent years, they are as ever present in the discussion of endogenous pain modulation(146), mood (93, 94) and consequently in labor (92).

In our study, where women were included prospectively and followed longitudinally during pregnancy, we confirmed prior results indicating an increase in plasma levels of BE during the third trimester (100, 147), but
failed to detect any difference in BE between the first and third trimester. Furthermore, our findings also indicated that low levels of BE at the end of pregnancy are associated with increased usage of second line analgesia.

A number of previous cross-sectional studies have assessed BE levels throughout pregnancy (95-99), but only two of them have used a longitudinal design (100, 101). In a small study comprising 10 pregnant women, Browning and co-workers reported gradually increasing levels of BE throughout the course of pregnancy, with the increase starting already at gestational week 16-20 (100). We were unable to replicate the findings of Browning and colleagues but, in line with two previous studies (101, 148), we found a subsequent increase during the third trimester. Given the substantially larger sample size of the present study, our estimates of BE levels throughout the course of pregnancy are more valid than prior cross-sectional and relatively small longitudinal studies.

The significant decrease of BE levels in gestational weeks 28 and 33 compared to gestational week 10 is in line with previous cross-sectional studies (97) and could possibly be due to the successive increase in blood volume of pregnant women, which is known to occur at the end of the second trimester. The subsequent increase of BE in late pregnancy might, in turn, be a result of increased stress at the end of pregnancy but could also be due to increased production of BE from placental tissue (101, 149).

Very few attempts have been made to relate BE levels at the end of pregnancy to clinical outcomes. The association between low levels of BE in plasma at gestational week 37 and increased use of second line analgesia is in agreement with a previous study by Sandman and colleagues (147), who reported that plasma levels of ACTH and BE increase in the third trimester of pregnancy and that these factors are co-released from gestational week 27-29 until delivery. However, this co-release pattern between ACTH and BE was disrupted in women who subsequently used conduction analgesia during labor (147). An even more pronounced elevation of endorphins has been described during labor (99, 103) and high BE levels during labor has been related to increased use of analgesia (94, 104). In addition, a negative correlation has observed between self-reported pain in labor and the postpartum BE levels (150). However, the relationship between low BE levels in gestational week 37 and increased use of second line labor analgesia is most likely mediated through factors, other than pain-modulation, of importance for labor analgesia use such as general level of physical activity during pregnancy (92, 151) or BMI (152-154).

While there appears to be an association between BE and major depression, it is rarely demonstrated as a simple deficit or excess (93, 155). In contrast, high levels of BE throughout pregnancy is associated with increased risk for postpartum depression (156), hypothetically mediated through a more pronounced BE withdrawal following delivery (27, 157, 158).
Studies of candidate genes; GCH1 and OPRM1

Two categories of potential genetic pain-perception pathways have been described, where neurotransmission modulators and mechanisms that affect inflammation are of main importance. The pain-related genes mainly discussed in the literature interact with specific receptors and signaling downstream of the receptor, modulation of opioid effects, metabolism and transport (119). The genes studied by us are pain-related genes known to be of interest in the clinic and they were specifically chosen as they have been proposed to be candidate genetic markers for pain perception and for individual sensitivity to analgesics.

The results from study II indicated that the pain-protective SNP combination of GCH1 may be of importance in a limited number of homozygous carriers during the initial dilation of cervix, but upon arrival at the delivery unit these women are more inclined to use second line labor analgesia. Intriguingly, the latter finding was partly replicated in paper IV where hetero- and homozygous carriers more often used second line analgesia than non-carriers.

Previous studies of the pain-protective SNP combination of GCH1 have been conflicting. The pain-protective effect appears to be most evident in patients with neuropathic rather than nociceptive pain. Tegeder and colleagues (134) suggested that the pain-protective SNP combination of GCH1 is associated with less pain following discectomy for persistent radicular low back pain and their results were later been reproduced both by themselves (140) and by others (141, 159). Further studies on the pain-protective SNP combination of GCH1 have been more discouraging. Kim and colleagues (160) found no association between rated pain severity after surgical removal of molar teeth and the pain-protective SNP combination of GCH1, and similar negative findings were obtained in patients with chronic pancreatitis (161). The pain-protective SNP combination of GCH1 may also have a role in chronic pain as it has been shown to delay the need of opioid treatment in cancer patients (162). The negative results in our study could be due to the fact that labor pain is mainly nociceptive or that pain modulating pathways affected by BH4 are not the primary ones during labor (134). A more speculative explanation is, however, that the pain-protective SNP combination of GCH1 has a bimodal effect. In situations with low-intensity pain, patients are less sensitive, whereas in situations with intense pain (as labor) the pain-protecting effect conferred by the SNP combination is altered. Yet another alternative speculation is that women with inherited pain protection have been less exposed and used to pain, and are thus more inclined to request analgesia when pain intensity exceeds a certain threshold.

In study III no association between homozygous OPRM1 G-allele carriers and labor pain-related behavior was detected. Previous research findings in
this field are diverse and the relevance of this polymorphism seems to be most evident in studies dealing with exogenously supplied opiates and pain perception (131, 163-165). This might partially explain our negative results but another reason for the lack of results in our study could be due to the somewhat blunt measures of pain perception and pain behavior that were used. Obviously, a difference in pain-related behavior depending on a genotype related to the function of the µ-opioid receptor is bound to involve treatment response to opioids. When this study was planned, additional outcome measures were incorporated, including the total sufentanil dose (or dose rate). However, this information turned out to be difficult to ascertain in many cases and could thus not be used. However, the relevance of positive findings in studies dealing with genetic polymorphisms in clinical settings is sometimes at risk of being overestimated (166, 167). The nature and range of genetic modulation of pain has thus far not been adequately addressed. Due to the limited number of patients in the studies (126, 127, 141, 159) performed as well as the limited number of genes and genetic variants investigated, the complete understanding of the relevance of genetic variants for personalized analgesic treatments is not ruled out.

More studies in this area are needed and hopefully collaborations in larger research groups or consortia might allow projects where a large number of patients can be included and projects as part of i.e. the HapMap project might improve the capacity. Complex physiological mechanisms such as pain modulation might be subject to GWA studies where 1 000 000 markers or more markers in the genome is analyzed instead of one specific SNP or haplotype. The advantage of GWA studies are that the influence of linkage disequilibrium is taken into consideration, since genes that are in close proximity of the studied gene also are observed (168). Thus far, the only genomewide approach in acute pain research (118) has focused on response to nonsteroidal anti-inflammatory analgesics in post-surgical pain (for GWA studies in chronic pain, see for instance (169, 170)). The study revealed a candidate SNP associated with analgesic onset, which was in linkage disequilibrium with a gene encoding a zinc finger protein (118). The study, however, included an extremely limited number of patients (n = 112), that it may be expected that further studies in this area is underway.

Obvious strengths in study II-IV are that the cohort is relatively homogenous study population where Caucasians dominate and the population-based design. In fact, the population based model is considered more robust when studying specific genes of interest, whereas case-control models are recommended only when a larger sample size is included (171). The homogenous population reduces the risk of incorrect genetic associations since population stratification is avoided (118).

There is a need for increased information on how labor pain is regulated, and possibly the 8 - 10% of all women who seem to endure labor without
Mental health during pregnancy and labor

The main finding of study IV was that antenatal self-rated depressed mood is associated with early arrival to the delivery department. Women with increased STAI-T scores reported higher self-rated pain prior to labor analgesia, which might indicate that antenatal anxiety is associated with increased self-rated pain prior to labor analgesia.

Previous studies regarding mental health and pregnant women have mainly focused on antenatal depression and perinatal outcomes such as preterm labor and low birth weight (172, 173). Depression and anxiety during pregnancy will also impact on a number of health-related behaviors and obstetric outcomes. For instance, it has previously been shown that antenatal depression and/or anxiety is associated with nausea, sick-leave, premature contractions, planned caesarean section, use of epidural analgesia, and self-experienced longer duration of labor (28). The current study also emphasizes that pain-related behavior and self-reported pain during labor may be affected by antenatal depressed mood and anxiety. Antenatal depressed mood was primarily associated with a less advanced stage of cervical dilation at arrival to the delivery department, but as discussed previously, this pain-related behavior may be confounded by factors which were not accounted for. We were not able to replicate prior findings that depressive disorders are associated with increased use of epidural analgesia or second line analgesia (28). Previous studies in this area have, however, had a different focus with obstetric complications as primary outcome in relation to depression or depressed mood, and relevant adjustment for factors contributing to use of labor analgesia (such as duration of labor) were not considered (26, 27).

Antenatal anxiety, measured as trait anxiety levels near term pregnancy did not influence any of the pain-related behaviors but women with high anxiety scores experienced more severe pain before labor analgesia was administered. This finding adds to previous literature suggesting a direct correlation between anxiety sensitivity and maximum pain during labor (24, 25). Although a few women reported very low scores for pain during labor, the clear majority indicated that pain was closer to worst imaginable pain and variability in pain scorings in this sample was limited. In addition, pain scorings were collected retrospectively, i.e. within 24 hours of delivery, and retrospective recall biases may have influenced the pain severity. Based on prior studies (24), it seems that anxiety sensitivity was the best predictor of maximum pain during labor. Anxiety sensitivity in a parturient refers to anxiety related symptoms due to belief (in this context) that the labor pains will harm her or her fetus. The relationship between anxiety sensitivity and any exogenous analgesia at all, should be specifically studied in future studies.
STAI-T has been explored by Curzik and Begic (18) who concluded that there is no reason to expect greater labor pain intensity in women with high levels of trait anxiety whereas it can be expected in women with high anxiety sensitivity. In our study we have not addressed the phenomenon of anxiety sensitivity.

A general concern with studies regarding depression and pregnancy is that the discrimination between psychosocial stress, subclinical depression and true depression is often inconsistent, i.e. women (especially of high socio-economic status) tend to report what is expected of them rather than how they feel (172). Thus, underreporting of depressed mood may be expected during pregnancy, why conclusions of labor pain related outcomes and depression may be hard to interpret. The issue of the pregnancy itself influencing the MADRS-S score was taken into consideration (174, 175), but given the relatively high socioeconomic status of the study group the two confounders might balance each other.
Smärtupplevelsen och användning av smärtstillande behandling under en förlossning varierar mellan olika kvinnor. Det finns en mängd förklaringar till detta; där tidigare förlossningserfarenhet, förlossningens duration, barnets vikt och psykologiska faktorer förefaller vara de mest inflytelserika. Den här avhandlingen har studerat såväl biologiska (effekter av kroppseget morfin (endorfin) och genetiska polymorfier) som psykologiska faktorers (depressions- och ångestsymtom) betydelse för smårtrelaterade beteenden under förlossningen. De två genpolymorfier som studerats härrör från specifika förändringar av basparen i OPRM1- respektive GCH1 genen och har tidigare associerats med bättre svar på smärtstillande behandling respektive minskad smärtkänslighet.

Det som kom att bli vår första studie rörde β-endorfin (BE) som är ett kroppsegetämne med en morfinliknande effekt vars frisättning påverkas av stress. Vi kunde visa att BE nivåerna var oförändrade under graviditet, men att de efter en sänkning i den andra trimestern ökade under graviditetens senare del. Låga nivåer av BE under graviditeten var dessutom associerat med större behov av smärtlindring under förlossningen.

Kvinnor som var bärare i dubbel uppsättning av den förändrade GCH1 genen kom till förlossningen med mer öppen livmoderhals jämfört med icke-bärare. Vidare efterfrågade de, i motsats till vad vi hade förväntat oss, i högre utsträckning minst två sorters smärtlindring. Motsvarande genetiska analys för OPRM1 genen påvisade ingen skillnad mellan bärare och icke-bärare avseende livmoderhalsstatus vid tid för ankomst till förlossningen eller behov av smärtlindring. Självrapporterad smärta påverkades inte av bärarskap av någon av genpolymorfierna. Våra studier bekräftar att förstföderskor och kvinnor med långdragna förlossningar har ett större behov av smärtlindring.

Det är känt att orsakerna till behov av smärtlindring och skillnader i smärtupplevelsen under förlossningen är multifaktoriella. För att ta reda på betydelsen av faktorer som nedstämdhet, ångest och kroppens eget morfin (endorfiner) för upplevelse av förlossningssmärta, gjordes fördjupade studier på området. Vår 4:e och sista studie rörde huruvida ångest och nedstämdhet under graviditeten kan ha betydelse för hur tidigt man söker i förloppet och behovet av smärtlindring. Kvinnor med mer uttalade depressionssymtom visade sig söka tidigare i förloppet med värkar än motsvarande kvinnor med låg poäng på depressionsskalan MADRS-S. Vidare skattar
kvinnor med hög poäng på ångestskalan (STAI-T) sin förlossningssmärta högre innan de får bedövning av barnmorskan än motsvarande kvinnor med låg eller ingen ångest. Vår slutsats blev att kvinnans psykiska hälsa under graviditeten har betydelse för smärtupplevelse men att det inte ensamt kan förklara eller förutse smärtupplevelsen hos enskilda kvinnor. Det behövs dock fortsatta studier inom fältet för att komma närmare en bättre förklaringsmodell av födande kvinnors olika behov av smärtlindring.
General Conclusions

Levels of BE in plasma remain unchanged between early and late pregnancy except for a nadir in the beginning of the third trimester. Low levels of BE in gestational week 37 is associated with increased need second line labor analgesia.

The pain-protective SNP combination of GCH1 may be of importance in homozygous carriers during the initial phase of labor. However these women are more inclined to use second line labor analgesia than corresponding heterozygous women and non-carriers.

There is no association between the OPRM1 A118G SNP regarding pain-related behavior measured as stage of cervical dilation on arrival to the delivery unit or use of any type of second line labor analgesia.

Depressed mood during pregnancy is associated with early arrival to the delivery department, whereas antenatal anxiety is associated with increased self-rated pain prior to labor analgesia.
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