Therapeutic and Substance Abuse Biomarkers in the Immediate Postpartum Period

by

Marta Baber

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Pharmacology and Toxicology
University of Toronto

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ABSTRACT

The aim of this thesis was to develop the use of therapeutic and substance abuse biomarkers relevant to the immediate postpartum period, to reduce the risk of adverse outcomes in mothers and their infants. A systematic review was conducted to identify the role of opioid pharmacogenetics in analgesic response among postpartum patients, and in adverse events in these patients and their breastfed infants. Following a thorough search of the literature, studies were screened for their eligibility, data was extracted, and study quality was evaluated. Collectively, the 17 studies included in the review revealed that CYP2D6, OPRM1 A118G, UGT2B7 C802T and ABCB1 G2677AT may contribute to postpartum analgesia or adverse events. A nested cohort study was performed to explore the role of codeine pharmacogenetics in a population of 98 post-cesarean women. Participants were genotyped and instructed to report their pain using the visual analog scale 1 h following each dose of codeine. The OPRM1 A118G and UGT2B7 C802T variants were found to predict codeine consumption in the cohort overall (P < 0.001) and among Caucasians (P = 0.001).
A liquid chromatography-tandem mass spectrometry method was developed and validated for the analysis of fatty acid ethyl esters (FAEE) in neonatal hair, for the purpose of identifying in utero alcohol exposure. In a cohort of 177 patients, baseline levels of FAEE were established in the hair of neonates born to women who drank one drink or less per week during the third trimester of pregnancy. The pharmacogenetic biomarkers examined in this thesis may assist with optimizing analgesia in the post-cesarean patient, and reducing the risk of adverse events in the mother-neonate pair. The development of FAEE analysis in neonatal hair as a biomarker for assessing prenatal alcohol exposure may reduce the risk of adverse outcomes in the developing child.
ACKNOWLEDGEMENTS

I attribute the completion of this work to the incredible individuals and groups that have supported me throughout my doctoral education.

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<tr>
<td>%CV</td>
<td>Percentage coefficient of variance</td>
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<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
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<td>ALDH</td>
<td>Aldehyde dehydrogenase</td>
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<td>ARBD</td>
<td>Alcohol-related birth defects</td>
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<td>BAC</td>
<td>Blood alcohol concentration</td>
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<td>BBB</td>
<td>Blood-brain barrier</td>
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<td>BZ</td>
<td>Benzoylecgonine</td>
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<tr>
<td>C-section</td>
<td>Cesarean Section</td>
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<tr>
<td>C6G</td>
<td>Codeine-6-glucuronide</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<tr>
<td>CDT</td>
<td>Carbohydrate-deficient transferrin</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CoA</td>
<td>Coenzyme A</td>
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<tr>
<td>CTZ</td>
<td>Chemoreceptor trigger zone</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<tr>
<td>EM</td>
<td>Extensive metabolizer</td>
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<td>EQC</td>
<td>External quality control</td>
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<td>EtG</td>
<td>Ethyl glucuronide</td>
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<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>EtS</td>
<td>Ethyl sulfate</td>
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<td>FAEE</td>
<td>Fatty acid ethyl esters</td>
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<td>FAS</td>
<td>Fetal Alcohol Syndrome</td>
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<td>FASD</td>
<td>Fetal Alcohol Spectrum Disorder</td>
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<td>GABA</td>
<td>Y-aminobutyric acid</td>
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<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
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<td>GGT</td>
<td>Y-glutamyltransferase</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>IM</td>
<td>Intermediate metabolizer</td>
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<tr>
<td>IS</td>
<td>Internal standard</td>
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<td>LC-MS/MS</td>
<td>Liquid chromatography-tandem mass spectrometry</td>
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<td>LOD</td>
<td>Limit of detection</td>
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<td>LOQ</td>
<td>Limit of quantification</td>
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<td>m/z</td>
<td>Mass-to-charge</td>
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<td>M3G</td>
<td>Morphine-3-glucuronide</td>
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<td>M6G</td>
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<td>MAST</td>
<td>Michigan Alcoholism Screening Test</td>
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<td>MCV</td>
<td>Mean corpuscular erythrocyte volume</td>
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<td>MOR</td>
<td>μ-opioid receptor</td>
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<td>MRM</td>
<td>Multiple reaction monitoring</td>
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<td>MS</td>
<td>Mass spectrometry</td>
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<td>MS/MS</td>
<td>Tandem mass spectrometry</td>
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<td>NRS</td>
<td>Numerical Rating Scale</td>
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<td>P-gp</td>
<td>P-glycoprotein</td>
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<td>pFAS</td>
<td>Partial Fetal Alcohol Syndrome</td>
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<td>PM</td>
<td>Poor metabolizer</td>
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<tr>
<td>QC</td>
<td>Quality control</td>
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<td>RP-LC</td>
<td>Reversed-phase liquid chromatography</td>
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<td>SRM</td>
<td>Selected reaction monitoring</td>
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<tr>
<td>UM</td>
<td>Ultra-rapid metabolizer</td>
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<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
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<td>VRS</td>
<td>Verbal Rating Scale</td>
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Chapter 1
INTRODUCTION

1.1. Statement of Problem

The immediate postpartum period is critical stage of care for the mother-child pair. The central healthcare goal during this time is to reduce the risk of adverse outcomes in both patients over the short and long-term.

In North America, nearly one third of all pregnancies are delivered via cesarean section (C-section) and the majority of these mothers are prescribed an opioid to manage postpartum pain (Farine and Shepherd, 2012; Hamilton et al., 2015). Moreover, it has been reported that roughly 30-40% of nursing mothers in North America are prescribed an opioid-containing medication for pain control, although some patients do not achieve effective analgesia (Health Canada, 2013). Inadequate pain management can make it difficult for a mother to care for her newborn and can increase her risk of thromboembolic events due to immobility. Moreover, following opioid consumption, there is a risk of adverse side effects in the mother, and in the neonate via breast milk (Lötsch et al., 2009a; Benyamin et al., 2008; Koren et al., 2006). Adverse events in the mother can lead to under-dosing and/or opioid discontinuation, which further contributes to inadequate analgesia. Furthermore, it has been demonstrated that intake of opioids while breastfeeding can cause infant drowsiness, central nervous system depression and even death (Madadi et al., 2008; Willmann et al., 2009; U.S. National Library of Medicine, 2015).

Among women who take opioids, there is a sub-population of opioid-addicted women. Within the last two decades, prescription opioid use has increased dramatically in North America (Greenfield et al., 2010). In the U.S., opioid abuse increased by 141% between 1992 and 2003 (CASACU, 2005), and some studies have suggested that opioid abuse is more common among women than men (Simoni-Wastila et al., 2004; Simoni-Wastila, 2000). It has been estimated that illicit drug use takes place in approximately 4% of pregnancies (PIDU, 2012) and women with opioid dependence issues begin taking opioids at an average age of 26, which coincides with peak reproductive age (DASIS, 2002). Opioid-abusing individuals or
Women tend to have a variety of co-morbidities and are prescribed multiple medications that may adversely interact with opioids (Brooner et al., 1997; Cicero et al., 2012; Jones et al., 2012). This may lead to drug-induced complications prior to pregnancy, during gestation and in the postpartum period. Neonates born to this sub-population may experience adverse post-natal effects associated with drug complications in the mother during gestation and breastfeeding.

One of the most prevalent combinations of polydrug use involves the simultaneous use of opioids and alcohol, therefore, women who abuse opioids are likely to abuse alcohol as well (McCabe et al., 2006). Exposure to alcohol during pregnancy is a major health concern in the neonate that postpartum medical professionals commonly encounter. Maternal alcohol consumption during gestation is associated with the development of Fetal Alcohol Spectrum Disorder (FASD). FASD is one of the leading known causes of mental retardation in North America with an estimated incidence of 1 out of 100 live births (Sampson et al., 1997). The annual economic burden is over $5 billion according to Canadian estimates (Stade et al., 2009). In most cases, a diagnosis relies on maternal self-reported history of alcohol use during pregnancy. Limitations associated with early identification of ethanol-exposed neonates preclude a timely diagnosis and medical intervention, which are necessary to reduce the risk of adverse outcomes.

1.2. Study Aims and Objectives

(1) To conduct a systematic review of the existing literature on genetic variability that (a) may affect analgesic response to opioids among postpartum patients, and (b) have been implicated in adverse events in these patients or their infants through exposure to opioids while breastfeeding;

(2) To investigate the role of codeine pharmacogenetics in a cohort of patients being treated for post-cesarean pain;

(3) To develop and validate a method that exhibits sufficient analytical sensitivity to quantify naturally occurring levels of fatty acid ethyl esters (FAEE) in the hair of non-alcohol exposed neonates;
To measure naturally occurring levels of FAEE present in the hair of neonates born to abstaining or mild social drinking mothers in the third trimester of pregnancy.

1.3. Rationale and Hypotheses

Substantial literature has been published implicating the role of pharmacogenetics in altering opioid pharmacokinetics and pharmacodynamics, which might explain differences in therapeutic outcomes (Smith 2008; Crews et al., 2012; Argoff, 2010; Ren et al., 2015; Somogyi et al., 2007; Lötsch et al., 2009b). Identifying genetic polymorphisms that may contribute to inter-individual differences in opioid analgesia in the mother and adverse events in the mother-child pair may assist in the development a genetically guided approach to postpartum pain management. These findings may also assist with preventing, identifying or treating opioid-related complications in an opioid-abusing population. It is hypothesized that there are genetic polymorphisms relevant to an opioid’s pharmacological pathway that are associated with analgesic effectiveness and adverse events in mothers and their neonates.

In order to address the limitations with the currently available methods for detecting prenatal alcohol exposure, FAEE – non-oxidative metabolites of ethanol – have emerged as biomarkers of prenatal alcohol exposure within the last two decades (Hungund and Gokhale, 1994; Bearer et al., 1992; Caprara et al., 2005a; Klein et al., 1999; Bearer et al., 1999; Bearer et al., 2003). Based on preclinical and clinical data, FAEE levels are expected to be elevated in the hair of neonates born to alcohol-exposed mothers relative to non-exposed neonates. In order to implement the use of hair testing to detect in utero ethanol exposure, baseline levels of FAEE need to be established among neonates who have not been exposed to alcohol. It is hypothesized that a suitable method can be developed and validated for the analysis of naturally occurring levels of FAEE present in infants born to abstaining or mild social drinking mothers during the third trimester.

Reducing adverse outcomes in the mother and neonate may be possible through the development of therapeutic and substance abuse biomarkers that can be utilized in the immediate postpartum period. Ultimately, the fetal/early neonatal period is one of the unique periods of life, which may be characterized by drug-induced birth defects and
xenobiotic toxicity through breastfeeding. It is unique in a sense that the exposure occurs through the maternal system. Both ethanol in pregnancy and opioids during breastfeeding represent substances with clinically significant outcomes.
Chapter 2
REVIEW OF LITERATURE

2.1. Post-Cesarean Pain

2.1.1. Definitions of Pain

Pain is a term that is commonly used to describe a process that exhibits significant heterogeneity in its pathophysiology, duration and subjective experience. As such, pain is not easily defined, and multiple definitions exist to describe its complex nature. A common definition of pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey and Bogduk, 1994).” A more recent definition of pain describes it as a physical sensation with characteristics that are commonly associated with tissue-damage, and which is accompanied by feelings of threat and unpleasantness (Price, 1999).

From an evolutionary standpoint, pain can be adaptive or maladaptive. Whereas adaptive pain functions to protect or promote recovery in an organism, maladaptive pain can be described as a disorder, and is associated with nervous system injury or dysfunction (Woolf, 2004). In terms of origin, pain is commonly subdivided into nociceptive and neuropathic types of pain. Nociceptive pain, which is commonly coupled with tissue damage, is derived from an intense or noxious stimulus (chemical, mechanical, or thermal) that is transmitted via nociceptors (sensory nerve cells) (Dubin and Patapoutian, 2010). Nociceptors are the nerve terminals of primary afferent fibers that respond to damaging stimuli via nerve impulses to the central nervous system (CNS) (Dubin and Patapoutian, 2010). Nociceptive pain can be subdivided into somatic and visceral pain, which originate from skin/deep tissue and internal organs, respectively (Kapur et al., 2014). Unlike nociceptive pain, neuropathic pain is not associated with stimulation of nociceptors. Rather it is pain caused by damage to or dysfunction of the peripheral or central nervous systems and it can persist without the presence of a disease (Woolf, 2004). It is a sequela of injury, disease, or malfunction of the nervous system. In addition to nociceptive and neuropathic forms of pain, there are other types, such as psychogenic and referred pain. Psychogenic pain refers to mental or emotional
factors that may cause or contribute to a painful sensation in the absence of physical tissue damage (Tyrer, 2006). Referred pain involves a sensation of pain in a location that is different from the origin of the painful stimulus (Arendt-Nielsen and Svensson, 2001). Phantom pain is a type of referred pain, in which painful sensations persist from a part of the body that has been lost, and from which neurotransmission no longer takes place (Kooijman et al., 2000).

In the practice of pain medicine, pain can be classified according to its duration, location, severity, origin, body system, mechanism, diagnosis, and treatment responsiveness (Smith, 2005). The International Association for the Study of Pain has developed a formal classification system, which categorizes pain based on anatomic region, organ system, temporal characteristics, intensity, duration, and etiology (Merskey and Bogduk, 1994).

2.1.2. Pathogenesis of Pain

Pain can be described according to four physiological processes: transduction, transmission, modulation and perception (Kitahata, 1993). The pain process originates in the peripheral nervous system, wherein noxious stimuli are sensed by primary afferent nociceptors that innervate the skin and deeper tissues in the body (Woolf, 2004). Transduction involves the transformation of noxious stimuli into electrical activity within the peripheral terminals of nociceptor sensory fibers (Woolf, 2004). These painful stimuli are relayed to the dorsal horn of the spinal column, and are ultimately transmitted to the brain. This process involves the transmission of action potentials from the peripheral terminals of nociceptor fibers to the central terminals of nociceptor fibers within the CNS (Woolf, 2004). Modulation or change in the sensory input can follow, such as its augmentation or suppression (Ossipov, 2012). Finally, perception takes place, whereby afferent input to the brain is deciphered, eliciting the sensory experience associated with pain. Processing and interpretation of the painful stimuli takes place in the somatosensory cerebral cortex region of the brain (Bushnell MC et al., 1999).

A number of ascending pathways are involved in transmitting nociceptive stimuli to the brain; however, there is a dominant pathway in which the majority of nociceptive neurons are situated (Aziz and Ahmad, 2006). The classic pain pathway involved in the
transmission of painful stimuli to the brain involves a three-neuron chain (Cross, 1994; Figure 1). The first neuron is located in the dorsal root ganglion, with one axon innervating tissue located in the peripheral nervous system, and the other axon synapsing with the second order neuron located in the dorsal horn of the spinal cord (Woolf, 2000). The axon within the dorsal horn synapses with the third order neuron located in the thalamus (Aziz and Ahmad, 2006). This synaptic connection ascends the spinothalamic tract. Finally, the third order neuron ascends to the somatosensory cortex (Woolf, 2000). Aside from the somatosensory cortex, it has been demonstrated that a large brain network or “pain matrix” is involved in the processing of acute pain (Melzack R, 1999). Neurons from the thalamus also project to the prefrontal cortex, anterior cingulate cortex, insular cortex, amygdala and cerebellum (Rosenquist, 2016). These structures become activated in the context of pain processing.

Figure 1. Simplified depiction of the three-neuron chain pain pathway (Reproduced with permission from Lamont et al., 2000)
There are also descending pathways that are involved in altering the ascending nociceptive information. Various parts of the brain are involved in the descending pathways, such as the hypothalamus, the periaqueductal gray matter, areas of the pons, and the somatosensory cortex (Rosenquist, 2016). These regions are involved in modulating afferent pain transmission. Descending fibers from the prefrontal cortex and the anterior cingulate cortex interact with opioids, noradrenergic and serotonergic systems, and synapse with nociceptive neurons within the dorsal horn of the spinal cord (Rosenquist, 2016). This can function to inhibit pain transmission and produce analgesia (Rosenquist, 2016).

2.1.3. Pain Assessment Scales

In order to assess pain in the context of research, a number of different pain scales have been used. Due to the subjective nature of pain, acquiring pain data typically relies on patient’s self-reporting. Three commonly used pain rating scales are the Verbal Rating Scale (VRS), the Numerical Rating Scale (NRS) and the Visual Analog Scale (VAS) (Williamson and Hoggart, 2005). The VRS is an ordinal scale that relies on the use of adjectives to convey pain intensities, such as no pain, minimal pain, severe pain, etc. These adjectives are commonly assigned numbers to assist with recording. The NRS is an 11, 21 or 101-point number-based scale with two extremes representing no pain and the most severe pain. This scale can either be administered verbally or graphically. The VAS is commonly presented as a 10 cm line with the endpoints of no pain and the most extreme level of pain. Intensity is indicated by placing a mark anywhere along the line and the pain score is determined by measuring from the “no pain” anchor to the location of the mark. All three scales are considered valid and reliable, although there are advantages and disadvantages associated with the use of each scale (Williamson and Hoggart, 2005).

2.1.4. Importance of Post-Cesarean Pain Management

Within developed nations, between 6.2-36% of all deliveries are C-sections (Betrán et al., 2007). Hence, health care providers routinely confront the challenge of adequately managing post-cesarean pain, while minimizing the risk of adverse events in the mother and infant (via breastfeeding).
Achieving adequate pain relief following a C-section is particularly important since women generally need to be able to effectively breastfeed and care for their newborn infants during this time. Although the duration of pain following C-section can vary significantly, moderate to severe pain typically lasts 48 hours (Bonnet et al., 2010). Inadequate pain control in the post-cesarean period can cause chronic pain. In a survey conducted among women who underwent C-section, 12.3% reported experiencing a level of pain that interferes with infant care 6 months later, and 6% reported persistent pain 1 year after surgery (Nikolajsen et al., 2004).

Effective pain management is also important because immobility secondary to postsurgical pain can increase women’s risk of thromboembolic events (James, 2009; Greer, 2012), which can be life threatening. Thromboembolism involves the formation of a thrombus (blood clot) in a blood vessel that is transported from its site of origin to block another blood vessel. The risk of venous thromboembolism, which includes deep vein thrombosis and pulmonary embolism, is highest during the postpartum period, with a relative risk of 20-fold compared to non-pregnant women (Carter and Gent, 2012). The risk is even greater among post-cesarean patients compared to those who deliver vaginally (Blondon et al., 2016). It is postulated that the risk for thromboembolic events increases during pregnancy due to augmented coagulability (Bremme, 2003), which has evolved to reduce the risk of bleeding in the event of miscarriage and during childbirth (James, 2009). Other factors that contribute to the risk of thromboembolism include reduced venous capacitance and outflow (Gordon, 2002; Macklon et al., 1997), vein injury during childbirth or surgery (Whitty and Dombrowski, 2002) and decreased mobility (James, 2009; Greer, 2012). Pain resulting from C-section can lead to reduced mobility, which can further increase the risk of a thromboembolic event. Hence, providing sufficient pain relief can be important for reducing this risk.

2.1.5. Post-Cesarean Pain Management Approaches

Immediately following surgery, patients are commonly placed on a regimen of opioids that are administered either systemically or neuraxially (Ismail, 2012). Within the US and many other countries, epidural or intrathecal morphine is the most commonly administered adjuvant therapy (Pan, 2006). Patients may also receive continuous or patient-controlled
epidural infusions of fentanyl, with or without local anesthetic (Pan, 2006). In the UK, diamorphine administered neuraxially is the preferred therapy for pain management following C-section (Rawal and Allvin, 1996). Among patients who receive general anesthesia during C-section, systemic opioids are most frequently prescribed, either by nurses on demand or via patient-controlled intravenous analgesia (Pan, 2006). Another approach to post-cesarean pain therapy involves prescribing smaller amounts of a combination of analgesics with different mechanisms of action. This is known as multimodal analgesia. The goal is to achieve synergistic or additive analgesia with an overall reduction in side effects (Pan, 2006). Opioid therapy can be combined with a non-steroidal anti-inflammatory drug or acetaminophen (Hyllested et al., 2002). Post-cesarean patients are commonly sent home with one or more analgesics, often including an opioid medication (Sujata and Hanjoora, 2014). Within Canada, roughly 30-40 percent of nursing women are prescribed an opioid-containing medication for postpartum pain management following C-section or episiotomy (Health Canada, 2013).

2.2. Opioids in Postpartum Pain Management

2.2.1. Variability in Opioid Responsiveness

It is widely recognized that within the general population there is substantial interindividual variability with respect to opioid response (Smith, 2008). However, common approaches to treating patients still tend to be generalized which can lead to sub-optimal therapeutic responses (Lötsch et al., 2009a; Benyamin et al., 2008; Crews et al., 2012). Difficulty with individualizing therapy is largely attributable to the plethora of clinical variables that play a role in opioid responsiveness. Some of these variables include sex, age, ethnicity, co-medication, comorbidities, history of opioid exposure and genetics. It has been demonstrated that women exhibit a greater analgesic response to opioids than men, however, the opposite has also been found (Fillingim, 2002; Cepeda and Carr, 2003). Women may be more likely to experience adverse events, such as sedation and respiratory depression, following opioid intake (Whitley and Lindsey, 2009). It has also been found that as patient age increases, opioid requirement decreases (Bellville et al., 1971). Macintyre and Jarvis 1996 found that age was the most significant predictor of morphine requirement.
following surgery and developed guidelines for opioid dosing according to patient age (Macintyre and Jarvis, 1996). Ethnic background has been demonstrated to give rise to differences in opioid consumption (Konstantatos et al., 2012). The underlying causes behind these apparent ethnic differences may be genetic or cultural in nature. Co-medication can also modulate opioid responsiveness if there is an overlap in drug metabolizing enzymes (Overholser and Foster, 2011). Altered pharmacokinetics as a result of taking two drugs that utilize the same enzyme can lead to differences in drug response. Comorbidities, such as a hepatic or renal insufficiency, can prolong the duration of action or produce a more prominent peak effect (Goodman and Limberd, 1996). Prior exposure to opioids can impact how patients respond to opioids in the future. For instance, an opioid-naïve patient might require a lower initial dose of an opioid relative to an opioid tolerant individual (Mercadante et al., 2006).

In a burgeoning age of personalized medicine, the role of genetics in opioid response has become a topic of keen interest among clinicians and researchers (Smith 2008; Crews et al., 2012; Argoff, 2010; Ren et al., 2015; Somogyi et al., 2007; Lötsch et al., 2009b). Within the last 10 years, substantial literature has been published elucidating the role of pharmacogenetics in modulating pain relief, and in explaining the occurrence of adverse events in individuals exposed to opioids. In particular, studies have found that genetic polymorphisms alter opioid pharmacokinetics and pharmacodynamics, which leads to interindividual variability in drug response (Argoff, 2010; Fagerlund and Braaten, 2001). The management of postpartum pain provides a unique clinical context to study the influence of genetics on opioid analgesia and adverse effects of opioid use.

2.2.2. Opioid-Induced Adverse Events in the Post-Cesarean Patient

Of key concern when treating postpartum pain is the potential for adverse side effects in the mother and in the neonate via breast milk. Adverse events in the patient can lead to under-dosing and opioid discontinuation, which can prevent adequate analgesia (Lötsch et al., 2009a). Side effects in the mother that can complicate opioid therapy include constipation, nausea, vomiting, pruritus, sedation and respiratory depression. The most common side effect associated with opioid intake is constipation, followed by nausea (Benyamin et al.,
Constipation results from the binding of opioids to µ-opioid receptors (MORs) that are involved in regulating motility and secretion within the gastrointestinal (GI) tract (Kurz and Sessler, 2003). This causes a reduction in gut motility, secretions and blood flow, which leads to constipation (De Luca and Coupar, 1996). Opioid action in the CNS can also affect autonomic outflow to the gut (Yuan and Foss, 2000). It is currently unknown whether this opioid side effect is primarily mediated by the central or peripheral nervous system (Benyamin et al., 2008).

Nausea and vomiting can be caused by a number of different mechanisms, including reduced GI motility, stimulation of the chemoreceptor trigger zone (CTZ) or enhanced vestibular sensitivity (McNicol et al., 2003; Sussman et al., 1999; Flake et al., 2004). Afferent signals that lead to the development of nausea are processed at the vomiting center within the medulla (Herndon et al., 2002). Impaired GI function caused by opioid intake can lead to the stimulation of mechanoreceptors, which in turn can cause symptoms of nausea and vomiting via signaling to the vomiting center (Herndon et al., 2002; Mannix, 1998; Guyton and Hall, 1996; Fallon, 1998). Opioids displace medullary neurons accepted by the CTZ, which normally function to inhibit neuronal firing within the CTZ (Herndon et al., 2002; Mannix, 1998; Guyton and Hall, 1996; Fallon, 1998). Removing the inhibitory input to the CTZ can increase neurotransmission from the CTZ to the vomiting center, leading to the development of nausea and vomiting (Mannix, 1998; Guyton and Hall, 1996; Fallon, 1998). Opioids can also bind to the vestibular apparatus in the temporal lobe, which is involved in detecting changes in equilibrium and providing direct input to the vomiting center (Mannix, 1998; Guyton and Hall, 1996; Fallon, 1998). Opioid binding to this structure can reduce the threshold for nausea, especially if drug binding is also taking place at the CTZ (Herndon et al., 2002).

The pathophysiology behind opioid-induced pruritus or itch has not been fully elucidated (Ganesh and Maxwell, 2007; Szarvas et al., 2003), however, a number of different mechanisms have been proposed. It has been demonstrated that binding of opioids to MORs in the brain and spinal cord can lead to pruritus (Ko et al., 2004; Thomas et al., 1993). Dopamine D₂ receptors and serotonin 5-HT₃ receptors may also play a role in the
development of pruritus (Horta et al., 2000; Horta et al., 2006; Horta and Vianna, 2003; Szarvas et al., 2003; Yeh et al., 2000). Other minor mechanisms that have been described, include the release of prostaglandins (Szarvas et al., 2003; Colbert et al., 1999) and histamine (Ganesh and Maxwell, 2007), and antagonism of glycine and GABA (Y-aminobutyric acid) receptors within the CNS (Krajnik and Zylicz, 2001).

There are several mechanisms that have been described to explain opioid-induced sedation or drowsiness. Opioid intake is thought to cause inhibition of central cholinergic activity and several inhibitory effects on cerebral activity (Slatkin and Rhiner, 2004). Opioids have been demonstrated to cause anticholinergic activity within multiple cortical and subcortical regions of the brain (Slatkin and Rhiner, 2004). The imbalance between cholinergic and dopaminergic systems is thought to contribute to sedation (Vella-Brincat and Macleod, 2007). The anticholinergic effects of morphine are thought to be mediated by the accumulation of glucuronidated morphine products, such as morphine-3-glucuronide (M3G) and the active metabolite morphine-6-glucuronide (M6G), particularly the latter (Vella-Brincat and Macleod, 2007). Moreover, the cholinergic systems play a role in consciousness and attention via cholinergic projections that extend from the basal forebrain to the thalamus and cortex (Slatkin and Rhiner, 2004; Vella-Brincat and Macleod, 2007). Acetylcholine functions in the domains of information processing and cortical arousal (Slatkin and Rhiner, 2004). A disruption in cholinergic pathways or a decrease in acetylcholine within these pathways is associated with CNS disturbances (Vella-Brincat and Macleod, 2007). It has also been postulated that opioids act to decrease sensory input, which can increase the likelihood of sleep (Martin, 1983). Several studies have demonstrated that opioids interfere with sleep-wake patterns by decreasing REM sleep (Kay et al., 1969; Furst, 1990; Arankowsky-Sandoval and Gold, 1995).

Opioid use is linked to respiratory depression, because respiratory control centers of the brain contain significant proportions of opioid receptors (Wamsley, 1983). Opioid receptors are also found in carotid bodies (Lundberg et al., 1979a; Wharton et al., 1980), in the vagi (Lundberg et al., 1979b) and within the lungs (Zebraski et al., 2000). Respiratory rhythm can be affected at low opioid doses (Lalley, 2003). The pre-Bötzinger complex in the
ventrolateral medulla and the retro-trapezoid and parafacial respiratory group (RTN/pFRG) located nearby form a coupled oscillator and are involved in generating a respiratory rhythm (Janczewski and Feldman, 2006; Onimaru and Homma, 2003). Binding of opioids to these structures leads to a slowed and irregular breathing rhythm (Mellen et al., 2003). Opioid action at the Kölliker-Fuse and parabrachial nuclei in the pons are also involved in modulating the respiratory pattern (Lalley, 2006). At high opioid doses, a reduction in tidal volume is observed, likely due to decreased tonic inputs from opioid sensitive chemoreceptors (Lalley, 2003). Opioid application to central chemoreceptors in the brainstem has been demonstrated to have a variety of depressant effects on respiration (Taveira da Silva et al., 1983). Opioid binding to peripheral chemoreceptors located in the carotid body leads to inhibition of its activity, which causes a decrease in the hypoxic ventilatory response (McQueen and Ribeiro, 1980).

2.2.3. Factors Associated with the Transfer of Opioids into Milk, and with Opioid-Induced Toxicity in Neonates

Since breast milk is considered the ideal food for growing infants, the majority of women in Canada (approximately 90%) breastfeed their babies (Statistics Canada, 2016). Given that nearly a third of all infants born in North America are born via C-section, up to 30% may be exposed to opioids through breast milk (Farine and Shepherd, 2012; Hamilton et al., 2015). The relative safety of a medication in the context of breastfeeding depends on the relative amount of drug that gets into the milk, the capacity of the neonate to metabolize the drug and the toxicity of the drug given a particular level of exposure. A number of factors play a role in the amount of xenobiotic that gets transferred into breast milk and the risk for opioid-induced toxicity in the neonate (Anderson, 1991).

Molecular weight, lipid solubility, protein binding and ionization are xenobiotic determinants of diffusion into milk (Berlin and Briggs, 2005). The ideal xenobiotic candidate for diffusion exhibits low protein binding, has a low molecular weight, is lipid-soluble and undergoes ionization upon entering breast milk (Hendrickson and McKeown, 2012). Opioids have a relatively small molecular weight and can readily diffuse across the alveolar membrane, where milk synthesis and secretion take place (Hendrickson and McKeown,
2012). However, lipid solubility is variable among specific opioids (Roy and Flynn, 1988), therefore, the ease with which specific opioids cross the membrane also varies. Given that breast milk has a greater lipid content than serum, lipid-soluble opioids can become “trapped” within the milk, leading to a buildup of opioids (Hendrickson and McKeown, 2012). Since opioids are weak bases, they are susceptible to ionization and ion trapping upon entering breast milk due to the fact that milk is slightly more acidic than serum (Hendrickson and McKeown, 2012). This can lead to the accumulation of opioids in milk at concentrations that are higher than in maternal circulation. Overall, opioids exhibit moderate protein binding, although there is significant variability within the opioid drug class (Hendrickson and McKeown, 2012). Taken together, this data suggests that opioids exhibit moderate diffusion capabilities. It should also be noted that during the first week postpartum, xenobiotics may pass into the milk more easily due to the fact that tight junctions do not effectively seal the interstitial space from the paracellular pathway and the mammary alveolus (American Academy of Pediatrics, 2001).

Maternal pharmacokinetic parameters also modulate the amount of xenobiotic that passes into breast milk. The amount of drug present in the maternal systemic circulation determines how much drug ultimately transfers. Induction or inhibition of enzymes involved in the metabolism of opioids can alter levels of drug in the serum (Hendrickson and McKeown, 2012). Modulation of enzymatic activity can be caused by genetic polymorphisms or co-administration of a drug that uses the same metabolizing enzyme.

This risk of opioid-induced toxicity via breastfeeding is also dependent on neonatal pharmacokinetic parameters. Neonates are particularly susceptible to opioid-induced adverse events, because they consume a larger volume of milk per kg of body weight compared to older infants (Ito and Lee, 2003). Absorption of xenobiotics tends to be slower in neonates compared to adults (Kearns et al., 2003). In terms of metabolic capacity, certain drug metabolizing enzymes are not expressed at birth. Whereas CYP2D6 activity increases rapidly following birth (Treluyer et al., 1991), CYP3A4 and UGT2B7 activities are not fully established until several months after birth (Lacroix, et al., 1997; McRorie et al., 1992). Renal function is immature in neonates (Kearns et al., 2003). The adult glomerular filtration rate is achieved at
3-5 months of age (Arant, 1978; Leake and Trygstad, 1977). The blood-brain barrier (BBB) is also not completely developed at birth (Mahmood, 2008). Moreover, a drug efflux transporter at the BBB, P-glycoprotein (P-gp), is not fully expressed at birth and undergoes postnatal maturation (Lam et al., 2015). Decreased enzymatic function, impaired renal clearance, and an immature BBB in neonates can lead to the accumulation of pharmacologically active opioids or metabolites within the body and brain. Therefore, newborns and premature neonates are particularly susceptible to the effects of narcotics. Body composition can also mediate the risk for opioid toxicity. Neonates have a lower body fat content than older infants. Therefore, intake of a lipid-soluble opioid in the former group is associated with a greater risk for drug accumulation in the brain (Carberry et al., 2010).

2.2.4. Opioid-Induced Toxicity in Neonates via Breast Milk

Maternal use of opioids during breastfeeding can cause infant drowsiness, CNS depression and even death (Madadi et al., 2008; Willmann et al., 2009; U.S. National Library of Medicine, 2015). In 2006, there was a case report of fatal CNS depression in a neonate that was breastfed by a mother that was taking codeine for post-cesarean pain and who was genetically predisposed to produce very large quantities of morphine (Koren et al., 2006). On post-natal day 13, the neonate was pronounced dead and postmortem analyses revealed a blood morphine concentration of 70 ng/mL. This concentration is approximately 32-fold higher than the typical upper limit blood morphine concentration found in breastfed neonates whose mothers are taking a similar amount of codeine. The death of the neonate was attributed to opioid overdose caused by exposure to toxic levels of morphine via breast milk. A systematic review was subsequently published examining whether maternal use of codeine causes CNS depression in breastfed infants (Madadi et al., 2008). The Naranjo Adverse Reaction Probability Scale was used to establish causality. A total of 35 infants with adverse drug reactions were reported among 6 different sources and codeine was identified as a definite cause of CNS depression in these infants. Furthermore, a dose-response relationship has been demonstrated between maternal dose of codeine and neonatal opioid toxicity (Madadi et al., 2009).

There is limited data relating to the effects of maternal morphine intake on
breastfeeding infants. There is a case report of a term infant who was hospitalized with apnea and bradycardia with cyanosis (Naumburg et al., 1987). The mother had been taking morphine for pain. More studies have been published on the effects of oxycodone intake on breastfed neonates. A study was conducted to investigate the rate of sedation among 50 neonates exposed to oxycodone via breast milk over the first 2 days postpartum (Seaton et al., 2007). None of the infants were found to be severely sedated and less than 4% were moderately sedated. Given that sedation was evaluated over the first 2 days following birth, it is unlikely that most neonates were exposed to levels of oxycodone that were high enough to lead to sedation (Seaton et al., 2007). A retrospective study examined the rates of CNS depression among neonates exposed to either oxycodone, codeine or acetaminophen during breastfeeding (Lam et al., 2012). It was found that the rates of CNS depression among neonates exposed to oxycodone and acetaminophen were 20% and 0.5%, respectively. Women who reported adverse events in their neonates took higher doses of oxycodone and experienced more side effects as compared to mothers of unaffected neonates who took oxycodone. Almost all women reported that side effects were no longer observed in their neonates after oxycodone intake ceased. There are also case reports that describe neonates who experienced serious adverse events following exposure to oxycodone via breast milk (Sulton-Villavasso et al., 2012; Timm, 2013). Although there are no studies that have examined the neonatal effects of maternal hydrocodone intake, there are two case reports of nursing mothers who took hydrocodone while breastfeeding and whose neonates experienced CNS depression (Bodley and Powers, 1997; Meyer and Tobias, 2005). With respect to maternal sufentanil intake during nursing, one study found that there were no differences in behavior or clinical effects between neonates whose mothers received epidural sufentanil before and after C-section for 3 days postpartum, and those born to mothers who only received the drug prior to delivery (Cuypers et al., 1995). There is a case report of a nursing mother who used a transdermal fentanyl patch for chronic back pain for two weeks in the postpartum period (Cohen, 2009). The neonate did not experience any adverse side effects following exposure to the opioid through breastfeeding.

Several guidelines have been developed to assist with the safe use of codeine among postpartum mothers who are breastfeeding (Kelly et al., 2013). These guidelines can apply to
the use of other opioids during breastfeeding either in whole or in part. Women are advised to be vigilant of their symptoms following opioid intake. CNS depression in the mother is associated with an increased risk for CNS depression in the neonate. Therefore, neonates should be checked by a physician for signs of CNS depression in the event that the mother experiences symptoms. Neonates should also be monitored for difficulty feeding and breathing, adequate weight gain, prolonged sleep or signs of limping. It is recommended that codeine intake is limited to four consecutive days, because use beyond this length of time can lead to significant accumulation of potentially toxic morphine levels in breast milk, which increases the risk for CNS depression in the neonate. In general, it is recommended that nursing mothers consume the lowest possible dosage of an opioid medication. Non-narcotic analgesics are recommended to manage post-surgical pain beyond four consecutive days of codeine consumption. Finally, women who have a duplication of the gene that encodes CYP2D6 produce large amounts of morphine following codeine intake and are strongly advised to avoid using codeine for postpartum pain relief.

2.3. Opioid Pharmacology

2.3.1. Classification of Opioid Receptors and Opioids

Due to its analgesic effects, opium use dates back to Ancient Egypt and Mesopotamia (Breasted, 1930; Booth, 1986). The term “opioids” refers to any compound that binds to an opioid receptor to produce an analgesic effect, whereas “opiates” refers to alkaloid compounds that are naturally derived from the environment and act like opioids endogenously (Trescot et al., 2008). The array of effects produced by opioids can be in part explained by their diverse molecular actions and affinities for various receptors.

There are several different opioid receptor subtypes that are named after their archetypical agonist. Mu receptors are named after the morphine agonist and are predominantly found within the medial thalamus and brainstem (Trescot et al., 2008). There are two subtypes of the MOR, μ1 and μ2, which are associated with the positive and negative side effects of opioid binding, respectively. Whereas μ1 effects include analgesia, euphoria and serenity, μ2 effects are linked to sedation, respiratory depression, pruritus, vomiting,
urinary retention and physical dependence (Trescot et al., 2008). Ketocyclazocine is an agonist for κ-receptors. These receptors are located in spinal cord, brainstem, limbic system and parts of the diencephalon (Trescot et al., 2008). Binding of an opioid agonist is associated with pain relief, respiratory depression, sedation, shortness of breath, physical dependence, euphoria and dysphoria (Trescot et al., 2008). Finally, δ-receptors are targets of agonist delta-alanine-delta-leucine-enkephalin. They are primarily situated in the brain (Trescot et al., 2008). The effects of agonist binding are not well known; however, it has been postulated that it leads to analgesia, psychotomimesis and dysphoria (Trescot et al., 2008).

The three opioid receptor subtypes are controlled by different genes; however, the basic structure of each receptor is the same. Opioid receptors belong to the G protein-coupled receptor family (GPCR), which are characterized by seven transmembrane helical twists, three intracellular and extracellular loops, an intracellular C-terminus and an extracellular N-terminus (Fossépré et al., 2014). Opioid receptors are expressed throughout the CNS, including the medulla locus coeruleus, periaqueductal gray area, nuclei of the solitary tract, substantia gelatinosa of the spinal cord and within cortical, midbrain and limbic regions (Al-Hasani and Bruchas, 2011; Simantov et al., 1977).

Opioids can be classified according to their source: endogenous, naturally-occurring in the environment, semi-synthetic and synthetic. Endorphins, enkephalins and dynorphins are three major classes of endogenously produced opioids, which bind to opioid receptors present throughout the CNS and peripherally, in response to noxious stimuli. Pro-opiomelanocortins, pro-enkephalins and pro-dynorphins are the precursors for endorphins, enkephalins and dynorphins, respectively (Koneru et al., 2009). Enkephalins are relatively selective for delta receptors, whereas endorphins and dynorphins are selective for μ-opioid and κ-receptors, respectively (Koneru et al., 2009). Aside from producing an analgesic effect, these compounds are also involved in thermoregulation, cardiovascular control, hormone secretion and can act as neurotransmitters (Trescot et al., 2008). The opiates codeine and morphine are alkaloids that are naturally derived from the poppy plant Papaver somniferum. Within the plant, morphine is naturally produced through codeine demethylation (Phillipson et al., 1976). Codeine is typically synthesized from morphine, given that morphine is more
abundant in the environment (Thorn et al., 2009). Within the body, codeine is a pro-drug that is converted back to morphine, which is involved in producing analgesia (Smith, 2009). Semi-synthetic opioids, such as hydrocodone and oxycodone, are derived from naturally-occurring opiates, including morphine and thebaine. Synthetic opioids are synthesized without the use of opiates. Fentanyl and sufentanil are examples of synthetic opioids.

The majority of opioids used therapeutically exert their therapeutic effect via binding to MORs. Opioids can also be categorized according to their potency, affinity and efficacy upon binding to a particular receptor. The potency of a drug refers to its activity in terms of concentration or amount of drug required to produce a defined effect (Waldman, 2002). A highly potent opioid is required in smaller amounts than a less potent opioid to produce a pre-specified biological response or level of activity. A drug’s potency is characterized according to its affinity for and efficacy at a receptor. Affinity is a measure of a drug’s ability to bind to a receptor or degree of attraction to a receptor (McCabe, 2004). An opioid that has a higher affinity for a receptor relative to another is more potent, assuming all other drug characteristics are the same. The efficacy of a drug refers to its ability to produce a maximum biological response upon binding to a receptor (Wermuth et al., 1998). All other properties being equal, the greater the efficacy of an opioid, the greater its potency. An oral morphine-centric comparison of opioid potency reveals that codeine exhibits 1/10 of the potency of morphine, hydrocodone has the same potency as morphine, and oxycodone, fentanyl and sufentanil are 1.5, 80 and 800 times more potent than morphine, respectively (Table 1) (Syrmis et al., 2014; Zacny and Gutierrez, 2009; CDC, 2014; Rolly et al., 1979).

The efficacy of an opioid is characterized according to its action at a receptor relative to an endogenous agonist. For instance, at the MOR, morphine and fentanyl are full agonists, whereas buprenorphine is a partial agonist. A partial agonist produces a fraction of the biological response produced by a full agonist. At low doses, the effects of a full and partial agonist are indistinguishable among healthy individuals (Center for Substance Abuse Treatment, 2004). The differences in their effects become apparent above a certain dose threshold, known as the ceiling effect. Partial agonists are subject to the ceiling effect, whereby above a specific dose, the analgesic response does not change despite the fact that
the receptors are not fully activated (Center for Substance Abuse Treatment, 2004). However, full agonists continue to elicit greater biological responses with a rising dose until the receptors are fully activated or the maximum effect is attained (Center for Substance Abuse Treatment, 2004). Therefore, full agonists are the most potent analgesics. Opioid antagonists, such as naloxone, have an affinity for the MOR, but lack efficacy. They can competitively inhibit the binding of opioid agonists due to their relatively higher affinity to the MOR (Center for Substance Abuse Treatment, 2004). The binding of an antagonist terminates the biological effects elicited by the agonist.

Finally, opioids can be categorized according to their structural class. There are four major chemical classes according to the Drug Enforcement Agency classification system: phenanthrenes, phenylpiperidines, benzomorphans and diphenylheptanes (Trescot et al., 2008). Phenanthrenes are known as the “prototypical” opioids and contain a 6-hydroxyl group that is associated with nausea and hallucinations. Codeine, morphine, hydromorphone, oxycodone, hydrocodone and oxymorphone, belong to this class of opioids. Phenylpiperidines include fentanyl, alfentanil and sufentanil. It is a synthetic class of opioids that contain a piperidine that is attached to a phenyl moiety. Chemical structures of various opioids are shown in Figure 2 (ChemSpider, 2015).
2.3.2. Mechanisms of Opioid-Induced Analgesia

The classic principle behind opioid-induced analgesia is that activation of opioid receptors leads to inhibition of neuronal firing, which impedes spinal cord pain transmission.

Figure 2. Molecular structures of opioids
The analgesic effects of endogenous and exogenous opioids can be understood at the level of the brain stem, spinal cord and peripheral nervous system (Young-McCaughan and Miaskowski, 2001). In the brain, opioids activate descending pain pathways that originate from neurons within periaqueductal grey matter, which in turn project to the nucleus raphe magnus in the medulla (Basbaum and Fields, 1984). Neurons from the medulla extend to dorsal horn neurons in laminae I, II and V of the spinal cord and inhibit neuronal firing (Basbaum and Fields, 1984). Within the spinal cord, opioids inhibit the transmission of painful stimuli to the brain by directly inhibiting neurons within the dorsal horn (Sabbe and Yaksh, 1990; Yaksh, 1981). Peripherally, opioids act at the level of pre- and post-synaptic membrane potentials at primary afferent synapses to prevent neuronal firing (Stein, 1993).

All opioid receptors are coupled to inhibitory G-proteins that dissociate into their subunits, Gα and Gβγ, subsequent to agonist binding (Al-Hasani and Bruchas, 2011). These subunits go on to act on a variety of intracellular effector pathways. Similar to other G-protein coupled receptors, agonist binding to an opioid receptor leads to Gα-mediated inhibition of adenylate cyclase, which causes a reduction in cyclic adenosine monophosphate (cAMP) production (Al-Hasani and Bruchas, 2011). A decrease in cAMP can lead to the inhibition of the inwardly rectifying cation current Ca^{2+}, which can reduce neuronal firing (Al-Hasani and Bruchas, 2011; Harrison et al., 1998). Inhibition of neuronal activity is also mediated via modulation of calcium and potassium ion channels. Inhibition or closure of voltage-dependent calcium channels occurs via binding of the Gβγ subunit directly to the channels (Zamponi and Currie, 2013). In order for neuronal firing to take place, extracellular calcium needs to enter the presynaptic terminal, which ultimately leads to the release of pain neurotransmitters, such as glutamate and substance P (Harrison et al., 1998). The binding of these compounds to receptors on the postsynaptic membrane can cause neuronal excitability, which can lead to neuronal firing (Harrison et al., 1998). Therefore, by inhibiting calcium conductance into the cell, pain neurotransmission is halted. A reduction in neuronal firing is also accomplished via activation of G protein-coupled inwardly-rectifying potassium (GIRK) channels (Lüscher and Slesinger, 2010). Gβγ subunit binding to channels leads to their activation or opening (Huang et al., 1995). An increase in potassium efflux leads to hyperpolarization of postsynaptic neurons, which inhibits an action potential and reduces the
transmission of painful stimuli (Lüscher and Slesinger, 2010; Ikeda et al., 2000).

![Diagram](image)

Figure 3. Mechanisms associated with a reduction in neuronal firing (Williams et al., 2001; Permission not required)

The analgesic effect of opioids is also associated with the production of dopamine, serotonin and norepinephrine. Opioids can bind to presynaptic receptors on GABA neurons, which functions to block the release of GABA within the ventral tegmental area (Trescol et al., 2008). Since activation of GABA is associated with suppression of dopaminergic activity, inhibition of GABA facilitates dopaminergic neurotransmission and the release of dopamine in the nucleus accumbens (Trescol et al., 2008). This increase in dopamine leads to a pleasurable effect. Opioids can also act as antagonists at NMDA receptors, which activates the descending norepinephrine and serotonin pathways, leading to a reduction in pain (Trescol et al., 2008).
2.3.3. Opioid Pharmacokinetics

Opioid drug characteristics, such as primary sites of biotransformation, protein binding, oral bioavailability, half-life, primary routes of elimination and potency relative to morphine are described in Table 1. The pharmacokinetic parameters are expressed according to adult values.

Table 1. Opioid characteristics

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Site of Biotransformation</th>
<th>Main Enzymes</th>
<th>Protein Binding</th>
<th>Oral Bioavailability</th>
<th>Half-life Elimination</th>
<th>Primary Routes of Elimination</th>
<th>Potency Relative to Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>Hepatic</td>
<td>CYP1D6, CYP3A4</td>
<td>7-25%</td>
<td>53%</td>
<td>2.5-3.5 hours</td>
<td>Urine and a small proportion through feces</td>
<td>0.1</td>
</tr>
<tr>
<td>Morphine</td>
<td>Hepatic</td>
<td>UGT2B7</td>
<td>10-35%</td>
<td>17.33%</td>
<td>2-4 hours</td>
<td>Urine and a small proportion through feces</td>
<td>1</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>Hepatic</td>
<td>CYP1D6, CYP3A4</td>
<td>36%</td>
<td>25%</td>
<td>3.3-4.4 hours</td>
<td>Urine</td>
<td>1</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Hepatic</td>
<td>CYP3A4</td>
<td>38-45%</td>
<td>60-87%</td>
<td>3.7 hours</td>
<td>Urine</td>
<td>1.5</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Hepatic and small intestine</td>
<td>CYP3A4</td>
<td>79-87%</td>
<td>~50% (Transmucosal Loænge)</td>
<td>2-4 hours (IV)</td>
<td>Urine and a small proportion through feces</td>
<td>80</td>
</tr>
<tr>
<td>Sufentanil</td>
<td>Hepatic and small intestine</td>
<td>CYP3A4</td>
<td>91-93%</td>
<td>78% (Buccal) 9% (Oral)</td>
<td>2.7 hours</td>
<td>Urine</td>
<td>800</td>
</tr>
</tbody>
</table>

References: UpToDate Codeine, 2016; UpToDate Morphine, 2016; UpToDate Hydrocodone, 2016; UpToDate Oxycodone, 2016; UpToDate Fentanyl, 2016; UpToDate Sufentanil, 2016; Polston and Wallace, 2016; Elliot, 2010; Labroo et al., 1997; Willis et al., 2015; Syrmis et al., 2014; Zacy and Gutierrez, 2009; CDC, 2014; Rolly et al., 1979

Codeine is primarily metabolized in the liver, with some metabolism also taking place within the intestines and brain (Ohno et al., 2008; Milne et al., 1996). Roughly 50-70% of codeine is glucuronidated to codeine-6-glucuronide (C6G) by UGT2B7 (Coffman et al., 1997), 10-15% is N-demethylated to norcodeine by CYP3A4 (Caraco et al., 1996), 0-15% undergoes O-demethylation to morphine by CYP2D6 (Yue et al., 1991) and up to 11% is metabolized to hydrocodone (Oyler et al., 2000). The mechanism and clinical effect associated with hydrocodone production from codeine is unknown (Smith, 2009). Whereas codeine, C6G and norcodeine have a similar affinity for the MOR, morphine’s affinity is roughly 200-fold, which translates into a significantly stronger analgesic effect (Smith 2009; Caraco et al., 1996). Morphine is glucuronidated to roughly 60% M3G and 10-15% M6G by UGT2B7 (Ohno et al., 2008; Lötsch et al., 1996). UGT1A1 may have role in the production of M3G and M6G, and
UGT1A8 may be involved in the production of M6G (Holthe et al., 2002; Ohno et al., 2008). M3G is an inactive metabolite of morphine, whereas M6G is believed to be 100-fold more potent at the MOR than morphine (Ohno et al., 2008). Therefore, the ratio of M6G to morphine is considered an important predictor of analgesic efficacy. Morphine is also metabolized to a small amount of normorphine, an active metabolite that is formed by N-demethylation via CYP3A4 (Mithers, 1961).

Oxycodone is mainly metabolized to noroxycodone by CYP3A4/5 via N-demethylation (Kaiko et al., 1996), which is further broken down into noroxymorphone (Chen et al., 1991; Lalovic et al., 2006). CYP2D6 is involved in the O-demethylation of oxycodone to form oxymorphone, which accounts for 13% of oxycodone metabolism (Lalovic et al., 2004). Oxycodone, noroxycodone and oxymorphone can also undergo reductive metabolism, which yields a variety of reduced metabolites (Lalovic et al., 2004; Lalovic et al., 2006). It was previously believed that that oxymorphone was the metabolite primarily responsible for analgesic efficacy due to its higher affinity for the MOR than oxycodone (Thompson et al., 2004). However, it has been demonstrated that inhibition of oxymorphone formation in rats and humans does not alter the effects associated with oxycodone intake (Cleary et al., 1994; Kaiko et al., 1996; Heiskanen et al., 1998). The analgesic effect of oxycodone consumption has been attributed to the parent form of the drug, with a minor contribution from its breakdown products (Lugo and Kern, 2004).

Approximately 5% of hydrocodone is N-demethylated to norhydrocodone by CYP3A4 (Hutchinson et al., 2004; Cone et al., 1978). In urine, 5-6% of the hydrocodone dose is recovered as hydromorphone, which is formed by O-demethylation of hydrocodone via CYP2D6 (Hutchinson et al., 2004; Otton et al., 1993; Cone et al., 1978). Hydromorphone undergoes glucuronidation by UGT2B7 to form glucuronide products, such as hydromorphone-3-glucuronide, an active metabolite (Wright et al., 1998; Coffman et al., 1998). Roughly 40% of the clearance of hydrocodone has been attributed to non-CYP pathways (Hutchinson et al., 2004). Metabolites that have been detected include dihydrocodeine, isodihydrocodeine, dihydromorphine and isodihydromorphine. Hydromorphone is believed to play an important role in the analgesic effect associated with
hydrocodone use, since it has a 4-6-fold higher potency for the MOR than hydrocodone (Thompson et al., 2004). However, the relative contribution of hydrocodone and its metabolites to the analgesic effect associated with hydrocodone intake has yet to be clearly established (Coller et al., 2009).

Fentanyl in its unchanged form is responsible for producing analgesia, whereas its metabolites do not exhibit clinically relevant activity (Schneider and Brune, 1986). Its metabolism takes place within the liver. CYP3A4 is the principal enzyme involved in the N-dealkylation of fentanyl to norfentanyl, the major non-toxic breakdown product (Feierman and Lasker, 1996; Labroo et al., 1997). Fentanyl is also metabolized to despropionylfentanyl, hydroxyfentanyl and hydroxynorfentanyl (Labroo et al., 1997). These metabolites make up less than 1% of fentanyl metabolism. Sufentanil is a significantly more potent structural analog of fentanyl (Rolly et al., 1979). It undergoes N-dealkylation and O-demethylation and CYP3A4 is the main enzyme involved in the major metabolic N-dealkylation pathway (Monk et al., 1988; Tateishi et al., 1996). The main metabolites of sufentanil are n-desalkylsufentanil and 0-desmethylsufentanil (Rosow, 1984). The extent to which sufentanil’s metabolites contribute to its analgesic efficacy has yet to be determined (Monk et al., 1988).

2.4. Role of Genetic Polymorphisms in Opioid Pharmacology

2.4.1. CYP2D6 and CYP3A5

Cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6) is the main enzyme involved in the metabolism of codeine (Purdue Pharma, 2013) and hydrocodone (Otton et al., 1993), and to a lesser extent, oxycodone (Purdue Pharma, 2014). With over 80 allelic variants and its wide spectrum of enzymatic activity, individuals are commonly classified according to a particular CYP2D6 phenotype (Crews et al., 2012). CYP2D6 is the key enzyme responsible for the analgesic effect of codeine (Dayer et al., 1988). This highly polymorphic enzyme converts codeine, a pro-drug, into morphine, the active metabolite that induces analgesia. Much of the variability seen in response to codeine therapy has been attributed to CYP2D6. The CYP2D6 poor metabolizer (PM) phenotype is associated with decreased morphine production compared with intermediate metabolizers (IMs), extensive metabolizers (EMs) and ultra-rapid
metabolizers (UMs), leading to a reduction or complete absence of pain relief (Fagerlund and Braaten, 2001). CYP2D6 PMs carry two non-functional alleles of the CYP2D6 gene, whereas CYP2D6 IMs possess either two reduced function alleles or a combination of a non-functional allele with a functional or reduced function allele. CYP2D6 EMs possess two functional alleles, while CYP2D6 UMs have a duplication of the CYP2D6 gene resulting in two or more functional alleles. CYP2D6 UM status is associated with 50% higher morphine plasma concentration relative to EM, which can increase the risk for adverse reactions, such as dizziness, sedation, nausea and respiratory depression, even at low doses of codeine (Kirchheiner et al., 2007; Gasche et al., 2004). Due to the variability in analgesic response and emergence of side effects following codeine intake, a couple of groups have developed drug treatment guidelines according to CYP2D6 phenotype (Crews et al., 2014; Swen et al., 2011).

The expression of CYP2D6 is encoded on chromosome 22. Although CYP2D6 is predominantly expressed in the liver, it is also highly expressed in the GI system, kidney and brain (Miksys, et al., 2005). Phenotypic frequencies of CYP2D6 vary according to ethnicity. The worldwide prevalence of the UM phenotype is 1-2%, however, it is more frequent among individuals of African, Oceanic, American and Middle-Eastern descent (8-40%) compared to Europeans and Asians (2-3%) (Sistonen et al., 2007). The PM phenotype is most commonly found in Caucasians (8%) and more infrequent among individuals of Asian and African descent (Sistonen et al., 2007; Bernard et al., 2006). The EM phenotype is the most prevalent worldwide (Sistonen et al., 2007).

Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5) is involved in the metabolism of opioids, such as fentanyl (Jin et al., 2005) and oxycodone (Lalovic et al., 2006). Polymorphism at CYP3A5 rs776746 (also known as the CYP3A5*3 variant) is associated with reduced enzymatic activity (Kuehl et al., 2001). This allelic variant may impact CYP3A5-dependent clearance of an opioid, leading to a higher drug plasma concentration (Lam et al., 2013; Takashina et al., 2012). The gene encoding the expression of CYP3A5 is present on chromosome 7. This enzyme is largely expressed in the small intestine and colon (BioGPS, 2016). The frequency of CYP3A5*3 has been found to be higher in white Canadians (93%) than in Zimbabweans (77.6%) (Roy et al., 2005).
2.4.2. *OPRM1* A118G and *UGT2B7* C802T

Opioids are able to provide an analgesic effect by binding to the MOR. Polymorphism at the opioid receptor, µ1, *OPRM1* A118G, has been associated with decreased receptor expression, which may reduce the overall efficacy of opioids (Mahmoud et al., 2011; Zhang et al., 2005). This variant leads to a substitution at amino acid position 40, from asparagine to aspartic acid. A number of studies have investigated the role of *OPRM1* A118G in analgesia and opioid-induced adverse events in the postoperative period. Two recently published meta-analyses collectively found that 118G carriers had a lower incidence of side-effects following opioid intake, reported more pain and required higher doses of opioid analgesics following surgery than non-carriers (Ren et al., 2015; Hwang et al., 2014).

The expression of the MOR is encoded on chromosome 6. As mentioned above, it is primarily expressed within the brain and spinal cord (Al-Hasani and Bruchas, 2011; Simantov et al., 1977). It has been reported that the G allele is present in up to 50% of Asians, but only up to 10-15% in Caucasians (Sadhasivam and Chidambaran, 2012).

UDP glucuronosyltransferase 2 family, polypeptide B7 (UGT2B7) is involved in the glucuronidation of drugs, to facilitate their excretion in urine. UGT2B7 converts morphine to M6G, an active metabolite that is 100-fold more potent than morphine at producing analgesia (Pasternak et al., 1987; Sullivan et al., 1989; Francés et al., 1990). Although M6G and morphine have similar binding affinities for the MOR (Abbott and Palmour, 1988; Chen et al., 1991; Mignat et al., 1995), M6G has been postulated to exhibit selective antinociceptive and agonist effects that morphine does not (Pasternak and Standifer, 1995). The *UGT2B7* C802T variant has been implicated in the increased production of M6G leading to increased analgesia. This variant causes a substitution at amino acid position 268, from histidine to tyrosine. The biological significance of the *UGT2B7* C802T variant in the context of opioid analgesia is unclear. It has been suggested that the T allele is associated with higher M6G/morphine plasma ratios (Sawyer et al., 2003), which is associated with improved analgesia (Portenoy et al., 1992). Conversely, it has been demonstrated that this variant is not associated with altered M6G/morphine ratios (Holthe et al., 2003) and that higher M6G plasma levels are not linked to enhanced analgesia (Lötsch et al., 1997).
The gene encoding the expression of UGT2B7 is present on chromosome 4. It is expressed throughout the body, including the liver, kidney, lower GI tract and brain (Zhang et al., 2016). The T allele is more commonly found in Caucasians (54%) than Asians (27%) (Lampe et al., 2000).

2.4.3. COMT polymorphisms

The catechol-O-methyltransferase (COMT) enzyme is involved in pain perception through the breakdown of catecholamines and interacts with the opioid receptor system (Pertovaara, 2006; Chen et al., 1993). Single nucleotide polymorphisms (SNPs) in the COMT gene have been associated with variability in pain sensitivity following surgery (Lee et al., 2011; Dai et al., 2010) and in analgesia following opioid intake (Rakvåg, et al., 2005).

The most commonly studied SNP is COMT rs4680 (also known at Val158Met). This variant is characterized by a substitution of valine for methionine at amino acid position 158, which reduces COMT enzyme activity by 3 to 4 times (Lotta et al., 1995). Val/Val, Val/Met and Met/Met subjects are associated with a high, intermediate and low COMT enzyme activity, respectively. Relative to high COMT enzyme activity, low COMT activity results in reduced breakdown of catecholamines, such as dopamine and epinephrine. Several studies have examined whether COMT enzyme activity can explain interindividual variability in pain perception and analgesia following opioid intake (Zubieta et al., 2003; Rakvåg, et al., 2005; Rakvåg, et al., 2008). Interestingly, it has been demonstrated that Met/Met individuals require lower doses of morphine than Val/Val individuals for cancer pain (Rakvåg, et al., 2005), despite the fact that it has been shown that Met/Met subjects generally have a lower pain threshold and higher affective pain ratings (Zubieta et al., 2003). This is reconciled by the fact that Met/Met subjects have a greater regional density of MORs within the brain, which might translate into more effective analgesia following opioid intake relative to Val/Val subjects (Zubieta et al., 2003; Rakvåg, et al., 2005). It has been postulated that the higher regional density of opioid receptors may be due to enhanced dopaminergic neurotransmission because of a build-up of dopamine caused by low COMT enzyme activity (Rakvåg, et al., 2008). Animal models have revealed that an increase in dopaminergic neurotransmission leads to a reduction of enkephalin peptides, which are naturally occurring
opioid peptides within the body (Steiner and Gerfen, 1998). A drop in enkephalin peptides within the CNS leads to compensatory rise in the regional density of MORs within the brain, which translates into better analgesic response following opioid consumption (Chen et al., 1993). An increase in reported hyperalgesia among Met/Met individuals may be due to stimulation of β2-adrenergic receptors caused by an increase in catecholamine levels (Khasar et al., 1999).

Together with COMT rs4680, synonymous variants COMT rs4818 and COMT rs4633 are in strong linkage disequilibrium, therefore COMT haplotypes have been established corresponding to low, average and high pain sensitivities (Diatchenko et al., 2005). Lower levels of COMT enzyme activity are associated with the average and high pain sensitivity haplotypes, whereas increased COMT enzyme activity is associated with the low pain sensitivity haplotype (Diatchenko et al., 2005). This is expected, given that low COMT enzyme activity is associated with increased stimulation of β2-adrenergic receptors, leading to an increase in pain sensitivity (Khasar et al., 1999). In the context of opioid analgesia, homozygote mutants of the COMT rs4818 polymorphism have been reported to consume less opioids than patients in the other genotypic groups (Henker et al., 2013); however, the opposite trend has been reported (Rakvåg et al., 2008).

Chromosome 22 encodes the gene involved in the expression of COMT. This enzyme is primarily expressed in the brain, liver, kidneys and blood (Matsumoto et al., 2003; Tenhunen et al., 1993; Lundström et al., 1995). The COMT rs4680 variant is most prevalent among Mexicans (63%) and Caucasians (54%) and less common among Africans (34%) and Asians (29%) (González-Castro, et al., 2013).

2.4.4. ABCB1 polymorphisms

Transporters present in liver cells, GI tract and the brain play a role in the clearance of opioids and their metabolites; therefore, they have the potential to influence a drug’s pharmacokinetics and pharmacodynamics. The analgesic action of opioids is made possible by their movement into the brain across the BBB. ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1) gene encodes P-gp, which is responsible for the efflux of drugs out of the
Select polymorphisms have been associated with decreased P-gp activity, which may lead to increased accumulation of drug in the brain. *ABCB1* C3435T, a synonymous polymorphism, has been linked to decreased P-gp expression and activity, which has been associated with enhanced analgesia following opioid consumption (Somogyi et al., 2007; Hoffmeyer et al., 2000). *ABCB1* G2677AT and *ABCB1* C1236T polymorphisms are in strong linkage disequilibrium with *ABCB1* C3435T (Fung and Gottesman, 2009) and have also been associated with reduced P-gp activity, leading to reduced efflux from cells (Sakurai et al., 2007; Llaudó et al., 2013). *ABCB1* G2677AT is a non-synonymous polymorphism that involves the substitution of serine for an alanine or threonine at amino acid position 893 and *ABCB1* C1236T is a synonymous polymorphism. In the literature, the roles of *ABCB1* G2677AT and *ABCB1* C1236T in opioid analgesia and adverse events have not been clearly established. Due to the strong linkage disequilibrium between *ABCB1* C3435T, G2677AT and C1236T, two haplotypes have been established - CGC and TTT, which account for high and low predicted P-gp activities, respectively (Kim et al., 2001; Tang et al., 2002; Salama et al., 2006). It has been demonstrated that among subjects who received intravenous fentanyl, those who carried the haplotype associated with reduced P-gp activity had statistically significantly higher rates of respiratory depression than subjects who carried the haplotype for high P-gp activity (Park et al., 2007).

The *ABCB1* gene is located on chromosome 7. This transporter is widely expressed throughout the body (Thiebaut et al., 1987). It is expressed on the luminal membrane of endothelial cells within the BBB, the apical surface of intestinal epithelial cells, on proximal tubular cells of the kidney and in many other tissues with an excretory, absorptive or “barrier” function (Beaulieu et al., 1997; Světlík et al., 2013). The TTT haplotype (low activity) is more common among Caucasians and Asians, whereas the CGC haplotype (high activity) is prevalent within the African American population (Kim et al., 2001; Tang et al., 2002).

A summary of the main polymorphisms relevant to opioid analgesia is described in the table below.
Table 2. Genetic polymorphisms pertinent to opioid analgesia

<table>
<thead>
<tr>
<th>Genetic polymorphisms</th>
<th>Relevance to opioid analgesia</th>
</tr>
</thead>
</table>
| CYP2D6 (numerous allelic variants; 4 phenotypic groups: PM, IM, EM, UM) | - Involved in the metabolism of several opioids  
- Converts codeine (pro-drug) to morphine (analgesic)  
- PM produces trace amounts of morphine (expect inadequate analgesia)  
- UM produces 50% more morphine than EM (expect adverse events) |
| OPRM1 A118G | - Opioids induce analgesia by binding to the mu-opioid receptor  
- Receptor variant 118G has been associated with decreased mu-opioid receptor expression  
- Expected to cause less effective opioid analgesia and a lower risk of side effects |
| UGT2B7 C802T | - Involved in the glucuronidation of opioids to facilitate excretion from the body  
- Metabolizes morphine into active M6G (a potent metabolite) and inactive M3G  
- 802T variant is implicated in the increased production of M6G, which is expected to lead to improved analgesia and an increased risk of adverse events |
| ABCB1 C3435T ABCB1 G2677A/T | - Encodes P-gp, which transports opioids out of the brain  
- 3435T is associated with reduced expression and activity of P-gp;  
2677A/T is similarly associated with decreased P-gp activity  
- This is expected to cause increased accumulation of opioids in the brain leading to improved analgesia and an increased risk of side effects |

EM: Extensive metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer; UM: Ultra-rapid metabolizer.

2.5. Alcohol Consumption During Gestation

2.5.1. Maternal Ethanol Pharmacokinetics

Limited research has been conducted on the effects of pregnancy-induced physiological changes on alcohol disposition. Following oral ingestion, ethanol is absorbed from the GI tract via passive diffusion (Cederbaum, 2012). Approximately 80% of the ethanol is absorbed from the upper parts of the small intestine and the remaining 20% is absorbed from the stomach (Swift, 2003; Kalant and Khanna, 2007). Absorption from the stomach is influenced by the gastric emptying rate, which may be prolonged in pregnant women (Levy et al., 1994). This may theoretically slow ethanol absorption from the stomach; however, there are no known clinically significant changes in the absorption of oral drugs (Levy et al., 1994; Magee and Koren, 2007). The rate of absorption is impacted by a number of other variables, including but not limited to presence of food in the stomach, rate of intake, presence of GI disease, co-ingestion of drugs and volume, type and concentration of alcohol ingested (Goldin and Marshall, 2016). The plasma concentration of ethanol is expected to be lower in pregnant
women compared to non-pregnant women due to the increase in total body water (Zelner and Koren, 2013). Ethanol is small, uncharged and water-soluble, so it easily distributes across the water compartments of the body (Norberg et al., 2003).

Metabolism is accomplished via oxidative and non-oxidative pathways. Only 5% of ethanol does not undergo metabolism prior to excretion through the breath, urine and sweat (Robinson, 2015). The majority of ethanol is metabolized in the liver through enzymatic oxidation, wherein ethanol is converted to acetaldehyde via alcohol dehydrogenase (ADH) (rate-limiting step) and subsequently metabolized to acetic acid by aldehyde dehydrogenase (ALDH) (Swift, 2003; Kalant and Khanna, 2007). Genetic polymorphisms in ADH and ALDH alter the rates of ethanol metabolism (Bosron and Li, 1986). Cytochrome P450 2E1 (CYP2E1) and catalase are also involved in the oxidative metabolism of ethanol to acetaldehyde, although their role is more minor compared to ADH (Zelner and Koren, 2013). In the context of chronic alcohol intake, CYP2E1 activity can be induced (Zakhari, 2006). The non-oxidative pathway of ethanol metabolism also plays a small part (Zelner and Koren, 2013). It involves the conjugation of ethanol with fatty acids, phospholipids, sulfate or glucuronic acid to form FAEE, phosphaditylethanol, ethyl sulfate (EtS) and ethyl glucuronide (EtG), respectively (Pawan, 1972; Zimatkin and Deitrich, 1997). In vitro and in vivo studies have demonstrated that the oxidative and non-oxidative pathways are linked. Inhibiting the oxidative pathway using inhibitors of ADH, cytochrome P450 and catalase, causes an increase in non-oxidative metabolism (Werner et al., 2002).

It generally takes roughly 1-3 hours to eliminate one alcoholic drink (12 ounces of beer, 5 ounces of wine, or 1.5 ounces of a distilled spirit) (Cederbaum, 2012; Koren, 2002). The rate of metabolism in the liver exhibits zero order kinetics, which signifies that it operates independently of blood alcohol concentration (BAC) and is linear over time (Holford, 1987). Studies in rats have revealed that there is an increased clearance rate in pregnant rats compared to non-pregnant rats matched according to weight, which may serve to reduce fetal exposure to alcohol (Badger et al., 2005). Moreover, ALDH and ADH activities were increased, which may serve to increase ethanol clearance and metabolism (Badger et al., 2005).
2.5.2. Fetal Ethanol Disposition

Animal and clinical studies have proven that ethanol readily crosses the placenta and rapidly equilibrates resulting in approximately equal BACs in the maternal and fetal compartments (Brien et al., 1983; Brien et al., 1985; Brien et al., 1987; Idanpaan-Heikkila et al., 1972). A study in healthy pregnant women revealed that the Cmax and AUC of ethanol is lower in the amniotic fluid compared to maternal blood; however, the ethanol elimination rate in the amniotic fluid is roughly half of the rate in maternal blood, suggesting that amniotic fluid serves as a reservoir to extend fetal exposure to ethanol (Brien et al., 1983). Fetal swallowing in the last two trimesters of pregnancy acts to further prolong exposure (Zelner and Koren, 2013).

Studies have revealed that first trimester fetuses exhibit limited oxidative metabolism due to low expression and activity of ADH enzymes (Hines and McCarver, 2002; Smith et al., 1971). The capacity for oxidative metabolism tends to rise with increasing gestational age (Smith et al., 1971). CYP2E1 activity is detectable in the fetal liver by the second trimester and also increases with gestational age, reaching 30-40% of adult expression levels one year following birth (Hines and McCarver, 2002; Vieira et al., 1996; Johnsrud et al., 2003). CYP2E1 expression has also been identified in fetal brains at 7-9 weeks gestation (Brzezinski et al., 1999). Given the limited availability of ADH and ALDH enzymes, its expression may facilitate the formation of reactive intermediates that cause toxicity (Brzezinski et al., 1999; Zelner and Koren, 2013). Studies have also found that CYP2E1 expression in the placenta has the potential to be induced by ethanol consumption suggesting that its variable expression may play a role in mediating fetal disposition to alcohol-related birth defects (ARBD) (Rasheed et al., 1997). In the non-oxidative pathway, enzymes necessary for FAEE synthesis are present early in gestation (Bearer et al., 1992; Bearer et al., 1995). Similarly, enzymes involved in the formation of EtG and EtS are present in the second trimester (Krekels et al., 2012). Interestingly, sulfotransferase expression and activity levels (involved in EtS formation) are present at adult levels or higher (Stanley et al., 2005). Although the non-oxidative pathway has a minor role in ethanol metabolism, it may have a compensatory role earlier in gestation when oxidative metabolism is less active (Zelner and Koren, 2013).
2.5.3. Risks Associated with Alcohol Intake

Alcohol consumption during pregnancy is associated with the development of FASD, an umbrella term used to describe a wide range of physical, behavioral and cognitive effects resulting from fetal alcohol exposure. Alcohol is the most widely used teratogen worldwide (Nevin et al., 2002). The teratogenicity associated with alcohol consumption in pregnancy is in part due to the fact that the fetus does not have full capacity to metabolize alcohol (Hines and McCarver, 2002; Smith et al., 1971). To date, no safe level of alcohol intake during gestation has been determined (CDC, 2016; Flak et al., 2014). However, there is controversy related to light-to-moderate drinking in pregnancy. While some studies have established that this level of drinking is not associated with problems (Falgreen Eriksen et al., 2012; Skogerbø et al., 2012; Underbjerg et al., 2012), other studies have shown that it may be associated with adverse behavioural and cognitive effects (D’Onofrio et al., 2007; Flak et al., 2014). Given that there is significant heterogeneity in terms of the reported fetal effects following any amount of alcohol consumption, most national and international health organizations recommend that women abstain from alcohol throughout pregnancy (CDC, 2016; Public Health Agency of Canada, 2012).

Undiagnosed and untreated FASD has been implicated in the development of secondary disabilities. Such disabilities can appear throughout a patient’s life and are believed to arise as result of complications from undiagnosed or untreated primary disabilities associated with FASD (Streissguth et al., 1996). It has been hypothesized that the interaction of mental and behavioural issues in combination with adverse environments can lead to the development of secondary disabilities (Streissguth et al., 1996). Such disabilities include mental health issues, problems at school, trouble with the law, inappropriate sexual behaviour, alcohol and drug addiction, issues with parenting and dependent living (O’Connor et al., 2002; Streissguth et al., 2004). Studies have demonstrated that early diagnosis of the disorder (before the age of 6 years) leads to a reduction in the incidence of secondary disabilities (Streissguth et al., 1996). An early diagnosis can enable early medical intervention, which may decrease the appearance and attenuate the course of secondary disabilities (Institute of Medicine, 1996; Streissguth et al., 1996).
2.5.4. Risk Factors for Fetal Alcohol Effects

Quantifying the risk of adverse effects in the fetus following alcohol intake is difficult; however, it has been demonstrated that the pattern of alcohol intake is predictive of alcohol-related effects. Interestingly, although it has been shown that the total volume of alcohol consumption is an important factor, the number of binge drinking episodes may be of greater importance (Maier and West, 2001). Binge drinking, which is defined as consuming 4 or more drinks in approximately two hours (for women), is associated with a high BAC (CDC, 2015). A high BAC is problematic because it is difficult for a fetus to eliminate large amounts of alcohol in a relatively short timeframe (Maier and West, 2001). Hence, as the number of binge drinking episodes in pregnancy increases, so does the risk for FASD.

Another important variable that contributes to the risk of fetal alcohol effects is timing of alcohol intake (Maier and West, 2001). Given the widespread consumption of alcohol among women of reproductive age and the fact that a large proportion of pregnancies are unplanned, it is fairly common for women to be exposed to alcohol prior to pregnancy recognition (Symon et al., 2016). Women may learn that they are pregnant right after missing their period. Therefore, exposure to alcohol may have occurred approximately two weeks following conception, a stretch of time commonly referred to as the “all-or-nothing period.” In the event that a xenobiotic crosses the placenta and affects the fetus, the consequence is either a spontaneous abortion or complete recovery of the embryo (Adam, 2012). The risk to the fetus is also dependent on whether there is exposure to alcohol in the first trimester. Organogenesis or structural development takes places throughout the first trimester and exposure to a teratogen during this critical period can lead to the development of major malformations in the fetus (Mitchell et al., 2011). Drinking beyond the first trimester may affect the physiological function of organs (Abel and Hannigan, 1995). The brain develops throughout pregnancy, making it susceptible to the effects of alcohol throughout pregnancy (Ornoy and Ergaz, 2010). Moreover, it has been shown that stopping alcohol consumption at any point in pregnancy can improve outcomes in the fetus. For instance, stopping before the end of the second trimester is associated with improvements in head growth (Coles, 1994).

There may also be genetic factors that contribute to the development FASD (Ramsay,
Genetic differences associated with altered alcohol metabolism can modulate the risk of fetal alcohol effects (Chernoff, 1980). Other risk factors associated with the development of FASD include higher maternal age, improper nutrition, little prenatal care, history of substance abuse, low education level and socioeconomic status, paternal drinking and having a previous child with FASD (Esper and Furtado, 2014; May and Gossage, 2011). The clinical outcomes associated with prenatal alcohol exposure are well documented in the literature; however, it may be difficult in some cases to establish a causal relationship due to confounding factors. For instance, in utero exposure to alcohol has been linked to poorer neurodevelopmental outcomes (Thompson et al., 2009). While alcohol exposure use may play a role, being raised in a chaotic or stressful environment might also contribute to this outcome (Ferguson et al., 2013). Environmental factors present in the lives of pregnant women with substance abuse problems may contribute to the some of the clinical outcomes commonly associated with prenatal alcohol abuse.

2.5.5. Mechanisms of Alcohol Teratogenesis

A number of hypotheses have been generated, and number of studies have been conducted to elucidate the mechanisms behind alcohol’s adverse effects on the developing fetus. It has been proposed that folate deficiency among pregnant women following alcohol exposure may lead to an increased risk of neural tube defects (Chen, 2008; Grewal et al., 2008). Given that alcoholics tend to exhibit reduced absorption of folate (Halsted et al., 2002) and that folate requirements increase in pregnancy (Greenberg et al., 2011), the likelihood of being folate-deficient is high among pregnant alcoholic women. Moreover, it has been established that subsequent to prenatal alcohol exposure, folate levels are lower in the body (McGuffin et al., 1975; McMartin et al., 1985) and abnormalities in the fetus are more severe in folate-deficient pregnancies (Greenberg et al., 2011; Safi et al., 2012). A number of pre-clinical studies have shown that folic acid supplementation during gestation is associated with the reduction in adverse outcomes, such as physical defects and growth impairment among offspring (Wang et al., 2009; Xu et al., 2008; Yanaguita et al., 2008). Ethanol consumption can also cause oxidative stress via the formation of free radicals or through the reduction of intracellular antioxidant capacity (Ornoy, 2007; Heaton et al., 2002; Kay et al., 2006).
Oxidative stress is associated with protein and DNA damage, and lipid peroxidation (Kay et al., 2006). Alcohol can also lead to increased production of prostaglandins, which are involved in fetal development (Ylikorkala et al., 1988). Administration of a prostaglandin synthesis inhibitor in mice prior to alcohol exposure reduced malformations by 50% compared to mice that received the inhibitor following alcohol intake (Randall and Anton, 1984. Alcohol has also been demonstrated to increase cellular apoptosis (Cartwright and Smith, 1995), impair neurogenesis (Cartwright and Smith, 1995; Heaton et al., 2002), disrupt the endocrine system (Haley et al., 2006) and alter gene expression (Vangipuram et al., 2008). It is likely that a combination of different mechanisms plays a role in alcohol’s teratogenicity (Cohen-Kerem and Koren, 2003).

2.5.6. FASD Diagnosis

FASD diagnosis typically involves a physical examination and a neurobehavioural assessment and is most accurate between the ages of 2 and 11 (Institute of Medicine, 1996). In Canada, Chudley et al. developed guidelines for the diagnosis of FASD (2005). Fetal alcohol syndrome (FAS) is the most severe form of FASD and is characterized by a triad of primary disabilities, including facial dysmorphology, growth restriction and CNS neurodevelopmental abnormalities (Sokol et al., 2003). In order to be diagnosed with FAS, an individual must demonstrate growth impairment, have 3 specific facial anomalies (short palpebral fissures, smooth philtrum and thin upper lip; Figure 4) and demonstrate impairment in 3 or more CNS system domains. This is the only form of FASD that does not require confirmation of maternal drinking during pregnancy. Hard and soft neurologic signs, brain structure, cognition, communication, academic achievement, memory, executive functioning, abstract reasoning, attention, adaptive behavior and social skills or social communication are the CNS system domains that are examined in order to assess functional CNS damage. Partial fetal alcohol syndrome (pFAS) diagnosis requires the presence of 2 facial anomalies, impairment in 3 or more CNS domains and confirmed maternal alcohol exposure. Presence of ARBD are established by confirmation of maternal alcohol use and the presence of one or more congenital defects, such as cardiac, skeletal and kidney defects, among others. At the lower end of this spectrum disorder, subtle behavioral and cognitive deficits may be present.
Alcohol-related neurodevelopmental disorder is characterized by impairment in 3 or more CNS system domains and confirmation of maternal alcohol consumption.

Figure 4. Discriminating facial features in individuals with FAS (NOFAS UK, 2016)

2.5.7. Prevalence and Incidence Rates of FASD

Within the United States, the estimated prevalence of FAS is 1-3 for every 1000 persons and the incidence of FASD is 9.1 per 1000 live births (Sampson et al., 1997; Chudley et al., 2005). These figures likely underestimate the prevalence and incidence of FAS and FASD because they are under-diagnosed (Sokol et al., 2003). Although there are no nation-wide statistics for Canada, there are prevalence estimates for several sub-populations. In a First Nations community within British Columbia, FASD prevalence was reported to be 190 per 1000 persons (Robinson et al., 1987). Two studies out of Manitoba reported an incidence of 7.2 per 1000 live births in the northeastern region (Williams et al., 1999) and a prevalence of FAS or pFAS of 55-101 per 1000 individuals in a First Nations community (Square, 1997). In Saskatchewan, the rate of FAS was estimated to be 0.589 per 1000 live births between 1988 and 1992, according to referrals to diagnostic clinics (Habbick et al., 1996). These incidence and prevalence rates are likely not reflective of Canada-wide statistics (Chudley et al., 2005).

It has been estimated that the annual economic burden of FASD in Canada is over $5 billion (Stade et al., 2009). Given that up to 60% of women worldwide drink alcohol at some point during their pregnancy, and given that the incidence of FASD is roughly 1 out of every
100 live births (Sampson et al., 1997; Koren and Nulman, 2002), early diagnosis and clinical intervention is critical to improve prognoses among those afflicted and subsequently reduce social and economic burdens.

2.6. Methods to Detect Prenatal Alcohol Exposure

2.6.1. Maternal Self-Report

One of the difficulties associated with FASD diagnosis is that verification of alcohol consumption during pregnancy is required unless there is a triad of features facilitating the diagnosis of FAS (Chudley et al., 2005). Yet, this triad only exists in roughly 10% of cases (NCALI, 1985; Smith et al., 1987). Developing methods to objectively and reliably ascertain prenatal alcohol exposure is imperative for improving identification and diagnosis.

Current diagnostic guidelines commonly rely on maternal-self reporting. There are a number of questionnaires available to screen for risk drinking, including the Michigan Alcoholism Screening Test (MAST), the CAGE questionnaire, TWEAK and T-ACE. MAST and CAGE questionnaires were originally established to assess alcoholism in male populations (Russell et al., 1996), whereas TWEAK and T-ACE were developed and validated to identify risky patterns of drinking in the obstetric population (Chang, 2001). TWEAK and T-ACE were adapted from MAST and CAGE, but designed to reduce feelings of guilt associated with drinking (Sokol et al., 1985; Bradley et al., 1998). For instance, the updated versions of TWEAK and T-ACE use indirect approaches to assess problematic drinking, because direct questions have been demonstrated to trigger denial, leading to under-reporting of alcohol intake among heavy drinkers (Sokol et al., 1985; Russell et al., 1996). Rather than asking about the number of drinks required to feel high, patients are asked about their level of tolerance or how many drinks they can hold (Russell et al., 1996). The benefits of administering these questionnaires are that they are relatively inexpensive, non-invasive and can be completed quickly (Floyd et al., 2006; American College of Obstetricians and Gynecologists, 2004). However, it is widely recognized that maternal self-reporting can demonstrate inadequate clinical sensitivity because of the stigma associated with prenatal alcohol use. A number of studies have revealed that maternal self-reporting exhibits low reliability in identifying
ethanol intake during pregnancy, since mothers tend to underreport their alcohol consumption (Ernhart et al., 1988; Stoler et al., 1998; Lange et al., 2014).

2.6.2. Traditional Laboratory Methods

Traditional laboratory methods used to ascertain alcohol exposure, such as blood and urine analysis are also plagued with limitations (Jones et al., 2010; Moller et al., 2010). Although urine analysis of ethanol is a highly sensitive and objective measure of alcohol consumption, the window of detection is relatively short (Moller et al., 2010). Testing needs to take place within 12 hours of a binge-drinking episode, otherwise there is a high risk of false-negative results (Moller et al., 2010). Employing the use of ethanol testing in urine to ascertain alcohol use in pregnancy would require that samples be collected frequently throughout gestation in order to generate a reliable history of alcohol use (Moller et al., 2010). This approach to substance abuse monitoring is time-consuming, costly, is subject to tampering (Fraser and Zamecnik, 2002) and relies on patient retention for follow-up appointments, which may difficult to achieve in a population that exhibits problematic drinking patterns. EtG can also be tested in urine, but up to 80 hours following alcohol consumption (Borucki et al., 2005). Although the duration of detectability for EtG is longer than ethanol, it is still subject to the same constraints in the context of assessing prenatal alcohol exposure. Moreover, EtG testing in urine can only be conducted at select laboratories worldwide (Moller et al., 2010). Similar to urine testing, alcohol testing in blood also has a limited time window of detection (12 hours or less), is subject to similar limitations and involves an invasive approach to monitoring alcohol intake in pregnancy (Jatlow and O’Malley et al., 2010; Bearer et al., 2004-2005). There are also methods to test cord blood, placenta, umbilical cord, amniotic fluid and neonatal urine, however, there is a narrow window of opportunity to collect these samples and they may be difficult to retrieve (Bearer et al., 2004-2005).

Blood-based biomarkers do not perform well in pregnant populations (Neumann and Spies, 2003) and may detect a level of alcohol consumption that is significantly over the threshold for adverse effects in the fetus. There are several biological markers commonly found in serum or plasma that can be indirectly used to assess chronic alcohol consumption
during pregnancy, including, but not limited to, carbohydrate-deficient transferrin (CDT), γ-
glutamyltransferase (GGT) and mean corpuscular erythrocyte volume (MCV). It should also be
noted that these are non-specific markers, as they can be elevated in other clinical conditions.

With a half-life of 15 days, CDT is involved in the movement of iron in the
bloodstream, and is found to be elevated in the serum of alcohol-abusing individuals (Joya et
al., 2012; Stibler et al., 1988). While studies have shown that it exhibits high specificity in
detecting chronic alcohol consumption, its utility in pregnant women may be limited since
CDT has been shown to increase throughout gestation, independent of alcohol consumption
(Conigrave et al., 2002; Allen and Litten, 2003). Moreover, Comasco et al. conducted a study
in pregnant women and found that CDT results were negative despite the fact that 12% of
women reported drinking alcohol during gestation (2012). These results suggest that CDT may
be more useful to detect heavier alcohol use in pregnancy; however, this consumption
pattern is likely to be above the threshold for adverse fetal effects. GGT is a glycoprotein
enzyme with a half-life of 4 weeks that rises in the serum levels of individuals with chronic or
excessive alcohol use (Joya et al., 2012; Orrego et al., 1985). Its action involves the transfer of
a glutamyl residue to an amino acid, peptide or water acceptor (Orrego et al., 1985). GGT
levels tend to decrease in pregnant women with increasing gestational age (Bacq et al., 1996).
These physiological changes should be taken into consideration when interpreting test
results. Excessive alcohol use is also associated with an increased MCV, reflected in the blood
3 months following consumption (Hasselblatt et al., 2001). Typically, it is used in combination
with other markers to detect alcohol use in pregnancy (Joya et al., 2012). When interpreting
MCV results, it is important to keep in mind erythrocyte volume can be increased up to 20%
throughout pregnancy and decreases rapidly following delivery (Bacq, 2000; Lund and

2.6.3. Maternal Hair Analysis

FAEE and EtG in maternal hair can serve as long-term biomarkers for chronic alcohol
consumption (Auwärter et al., 2001; Pragst et al., 2001). There are three models that have
been proposed to explain how FAEE are incorporated into hair. FAEE are primarily introduced
into hair through soaking in sebum, which contains FAEE that were synthesized in sebum
glands (Pragst and Yegles, 2006; Auwärter et al., 2001). It has been postulated that they may also be incorporated from the bloodstream and via synthesis of FAEE in the basal cells of the hair roots or surrounding tissues (Pragst et al., 2001). EtG is mainly incorporated into hair from the bloodstream and to a minor extent from sweat (Schummer et al., 2008; Schräder et al., 2012). Unlike blood testing, maternal hair analysis is a non-invasive approach to ascertaining prenatal ethanol intake. Since hair grows approximately 1 cm per month, hair can be segmented to determine patterns of alcohol consumption retrospectively (Pragst and Balikova, 2006).

EtG and FAEE levels can be assessed alone or in combination in the evaluation of chronic excessive alcohol consumption, which is defined as an average intake of 60 g or more of pure ethanol per day over the span of several months (Kintz, 2015). Hair FAEE and EtG are deemed to be effective qualitative markers of excessive ethanol use due to the fact that they do not exhibit a strong dose-response relationship (in terms of degree of consumption and levels measured in the hair) (Pragst, 2015). The cumulative concentration of four FAEE (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) is determined in the assessment of chronic excessive alcohol use (Auwärter et al., 2001; Pragst et al., 2001). Molecular structures of four FAEE analytes are pictured in Figure 5 (ChemSpider Draw, 2015). These four FAEE are analyzed because they have been found to be the most abundant relative to other FAEE in the hair of alcoholics. Hair is typically segmented to 0-3 cm or 0-6 cm hair segments prior to analysis (Kintz, 2015). A cumulative FAEE concentration over 0.5 ng/mg in the 0-3 cm proximal segment or over 1.0 ng/mg in the 0-6 cm proximal segment is strongly suggestive of chronic excessive alcohol consumption (Kintz, 2015). For EtG, a concentration equal to or greater than 30 pg/mg in 0-3 or 0-6 cm proximal segments is strongly indicative of chronic and heavy alcohol intake (Kintz, 2015). Strict abstinence is unlikely with FAEE and EtG concentrations greater than 0.2 ng/mg and 7 pg/mg in a 0-3 cm segment, respectively (Kintz, 2015). Given that FAEE and EtG can be quantified up to 6 cm from the root, a history of prolonged and heavy alcohol consumption in the second and third trimesters of pregnancy can be ascertained.
There are several limitations associated with FAEE and EtG analysis in maternal hair. First, cosmetic treatments and thermal hair straightening tools can modulate the levels of FAEE and EtG in the hair (Kintz, 2015; Ettlinger et al., 2014). The concentration of EtG can be reduced as a result of dying, bleaching and perming hair, which can lead to false-negative results (Suesse et al., 2012; Kerekes and Yegles, 2013). These hair treatments can also influence FAEE levels (Hartwig et al., 2003). Second, FAEE are formed in the presence of ethanol. Therefore, the use of hair products that contain ethanol, such as hairspray, gels, and hair lotions, can lead to increased levels of FAEE (Hartwig et al., 2003; Gareri et al., 2011). This can produce false-positive results. EtG levels are not impacted by the use of ethanol-containing hair products (Martins Ferreira et al., 2012). Given the widespread use of hair-care products, thermal hair tools and cosmetic treatments within the female population, FAEE and EtG test results may often be compromised by these factors (Gareri et al., 2014).

2.6.4. FAEE Synthesis, Degradation, and Transportation

FAEE are a family of non-polar neutral lipids that are comprised of more than 20 compounds that are synthesized in the presence of ethanol (Laposata et al., 1990; Bakdash et al., 2010). FAEE can be formed with numerous substrates and through the action of various enzymes. In the cytosol, an esterification reaction is catalyzed by FAEE synthase between free
fatty acids and ethanol (Best and Laposata, 2003). The carboxylic acid on the long aliphatic
chain of the fatty acid combines with ethanol to form FAEE and water. FAEE and Coenzyme A
(CoA) are the products resulting from the transesterification of fatty acyl-CoA and ethanol,
which is accomplished by the enzymatic activity of acyl-CoA ethanol O-acyltransferase (Best
and Laposata, 2003). There are also non-FAEE specific enzymes involved in the synthesis of
FAEE, such as carboxylesterase, cholesterol esterase, triglyceride lipase and lipoprotein lipase
(Best and Laposata, 2003; Tsujita and Okuda, 1994; Lange, 1982; Tsujita and Okuda, 1992).
These enzymes catalyze the formation of FAEE in the presence of ethanol and either
triglycerides, phospholipids, free fatty acids and cholesteryl esters (Zelner et al., 2013). It has
been shown that circulating FAEE in the blood following alcohol intake are primarily formed
by the action of lipoprotein lipase (Chang, 1997; Tsujita and Okuda, 1994). The metabolism of
ethanol is summarized in Figure 6. Levels of FAEE in the body are also influenced by FAEE
hydrolysis. It has been demonstrated that FAEE are rapidly degraded by cellular elements in
the blood and that cell-free plasma possesses limited hydrolytic activity (Saghir et al., 1999).
The white blood cell exhibits the highest capacity for FAEE catabolism (Saghir et al., 1999). It
has also been shown that FAEE hydrolytic activity is highest in the liver and pancreas, whereas
the heart and adipose tissue have the lowest activity (Diczfalusy et al., 2001). Within the cell,
FAEE transport is believed to be accomplished, at least in part, by a liver fatty acid binding
protein (Kabakibi et al., 1998). In the extracellular environment, FAEE are primarily
transported by lipoproteins and albumin (Bird et al., 1997).
Differences in FAEE species and their relative concentrations throughout the body can be explained by variability in enzymes involved in FAEE synthesis and breakdown, diverse fatty acid substrates and variable presence of carriers involved in the transport of FAEE (Best and Laposata, 2003). FAEE levels can also be impacted by the presence of lipid binding proteins, such as albumin, which can bind to fatty acid substrates thereby preventing FAEE synthesis (Kaphalia and Ansari, 2001).
2.6.5. FAEE as Mediators of Toxicity

After chronic alcohol abuse, FAEE are eliminated from the body according to two-phase kinetics. The primary and terminal half-lives are 3 and 11 hours, respectively (Doyle et al., 1994). Among heavy drinkers, FAEE are detectable in blood up to 99 hours following a drinking episode (Borucki et al., 2007). In addition to serving as biomarkers for alcohol exposure, FAEE may also be mediators of toxicity associated with alcohol consumption. It has been proposed that FAEE can uncouple oxidative phosphorylation, decrease cell proliferation and protein synthesis, incorporate into and cause disordering of organic bilayers, increase the fragility of lysosomes, and lead to the accumulation of lipid droplets (Pragst et al., 2001; Best and Laposata, 2003). Among individuals that have alcohol-induced tissue organ damage, it is believed that FAEE play a role given that they have been found to accumulate in various tissues that are particularly susceptible to the effects of alcohol (Laposata and Lange, 1986). FAEE accumulation was detected in the pancreas, heart, liver, brain and adipose tissue of individuals who suffer from an alcohol-related disease (Laposata and Lange, 1986). Conversely, little to no FAEE were found in organs that are not commonly affected by alcohol (Laposata and Lange, 1986). In vitro work has revealed that FAEE may be responsible for alcohol-induced damage to the liver (Szczepiorkowski et al., 1995). Incubation of HepG2 cells with FAEE is associated with a reduction in cell proliferation, protein synthesis and changes in cell morphology (Szczepiorkowski et al., 1995). In vitro and in vivo studies in animals have demonstrated that FAEE cause pancreatic damage (Werner et al., 1997; Haber et al., 1993; Ponnappa et al., 1994). FAEE are also linked to myocardial, mitochondrial and CNS injury (Bora et al., 1996; Lange and Sobel, 1983; Hungund et al., 1988; Gubitosi-Klug and Gross, 1996). Interestingly, elevated FAEE in meconium have been associated with FASD-related effects. It has been found that high FAEE levels are linked to deficits in executive functioning, cognitive ability, mental development and psychomotor performance (Peterson et al., 2008; Min et al., 2015; Noland et al., 2003). Whether FAEE play a role in the development of FASD will need to be elucidated in future studies.
2.6.6. FAEE – a direct biomarker of in utero alcohol exposure

In the last two decades, a number of animal and clinical studies have established FAEE, non-oxidative metabolites of ethanol, as effective biomarkers of fetal alcohol exposure. Unlike ethanol, FAEE persist in the body long after alcohol consumption has taken place and do not cross the placenta (Chan et al., 2004). Therefore, levels of FAEE found in the neonate can serve as an objective measure of exposure during pregnancy.

In an animal study conducted in rats, alcohol intake by pregnant rats was associated with the accumulation of FAEE in both maternal and fetal organs (Hungund and Gokhale, 1994). Another study revealed significant levels of FAEE present in fetal tissues and placenta tissues following alcohol administration to pregnant mice (Bearer et al., 1992). It was also found that human and mouse placentas possess significant FAEE synthase activity and FAEE can persist in placentas for 7 days (Bearer et al., 1992). A preclinical study conducted in guinea pigs revealed that chronic exposure to alcohol in utero is associated with significantly higher levels of FAEE in pup hair compared to non-exposed pups (Caprara et al., 2005a). The mean cumulative FAEE concentration measured in the hair of exposed pups was more than 15-fold higher compared to controls. These results suggest that FAEE levels in neonatal hair may be a promising marker to ascertain prenatal alcohol exposure.

A number of clinical studies have also been conducted to determine the utility of FAEE in meconium as a biomarker of gestational alcohol exposure. A case report described the testing of a meconium sample of a neonate born to a mother who self-reported drinking throughout her pregnancy (Klein et al., 1999). The total FAEE concentration was roughly 32-fold higher than the mean total FAEE concentration derived from 3 neonates whose mothers abstained from alcohol during gestation. In a clinical study conducted by Bearer et al., ethyl oleate levels in meconium were positively correlated with self-reported maternal drinking (2003). Ethyl oleate concentrations were most strongly related to average number of ounces of absolute alcohol consumed per day and drinking in the second and third trimesters. Using a cut-off value of 32 ng/g, the sensitivity and specificity for ascertaining alcohol consumption in pregnancy was 84.2% and 83.3%, respectively. The same study group previously reported an association between ethyl linoleate concentration in meconium and maternal drinking.
(Bearer et al., 1999). Women who consumed 1 or more drinks/week in the third trimester were distinguished from women who denied use, at a sensitivity and specificity of 72% and 51%, respectively. Similarly, Ostrea et al. found that ethyl linoleate and ethyl arachidonate concentrations in meconium demonstrated high specificity for detecting in utero alcohol exposure (2006). The prevalence of fetal alcohol exposure in Barcelona, Spain was assessed via FAEE analysis in meconium (Garcia-Algar et al., 2008). It was found that among alcohol-exposed neonates, ethyl palmitoleate and ethyl oleate levels were the highest and correlated most strongly with total FAEE concentration. Several other prevalence or incidence studies have been conducted in various populations using FAEE analysis in meconium (Moore et al., 2003; Bryanton et al., 2014; Gareri et al., 2008). Klein et al. were the first to report the analysis of FAEE in the hair of a neonate born to a mother who reported drinking during gestation (2002). FAEE were successfully quantified in neonatal hair.

2.6.7. FAEE Analysis in Meconium

FAEE can be measured in meconium to ascertain fetal ethanol exposure during the second and third trimesters (Ostrea et al., 1994; Miller and Holzel, 1974). Meconium, the first bowel movement of a newborn infant, is a useful matrix to detect in utero exposure to a wide range of xenobiotics. The two main mechanisms of drug deposition into meconium are through bile and through fetal swallowing of amniotic fluid containing drugs excreted through urine (Ostrea et al., 1989). Fetal swallowing is considered to be the main mechanism by which drugs are concentrated in the meconium (Ostrea et al., 1994). Once deposited in the fetal intestine, meconium preserves the xenobiotics deposited within it (Abusada et al., 1993). Analysis of meconium provides a direct and objective assessment of fetal drug or alcohol exposure, since FAEE do not cross the placenta (Chan et al., 2004). It has been proposed that drug use following approximately 12 weeks of gestation is reflected in meconium, since this is the period at which fetal swallowing begins (Ostrea et al., 1994; Miller and Holzel, 1974). Meconium analysis has been conducted in early-gestation human fetuses beyond the first trimester. Ostrea et al. discovered the presence of cocaine in the meconium of a 17-week old fetus (1994).
Typically, meconium collection involves scraping at least 0.5 g of specimen from the soiled diaper and placing it into a specimen collection container (Gareri et al., 2006). Studies have shown that meconium continues to be evacuated up to 125 hours following birth; however, it should ideally be collected within 24 hours (Gourley et al., 1990; Zelner et al., 2012). Sample collection after the first day of life can lead to false-positive test results for FAEE due to contamination of post-natal stool with dietary components or ethanol-producing microorganisms (Zelner et al., 2012).

Unlike meconium, neonatal hair is not subject to the same collection constraints and can be conveniently collected several weeks to months after birth (Gareri and Koren, 2010). There are a number of studies reporting the analysis of xenobiotics in neonatal hair to ascertain fetal exposure during the third trimester (published reviews: Lozano et al., 2007; Gray et al., 2007; Joya et al., 2012).

2.7. FAEE Analysis in Neonatal Hair

2.7.1. Hair Physiology and Fetal Hair Growth

Hair is an epidermal outgrowth that is composed of 65-95% protein (keratin), 1-9% lipids, 0.1-5% pigments and small amounts of water, polysaccharides and trace elements. Hair is made up of two major cell types (Harkey, 1993). The cuticle, which is the outermost layer of the hair shaft, is made up of overlapping scale cells (Kronstrand and Scott, 2006). The cortex is the layer underneath the cuticle and it is characterized by spindle-shaped cortical cells (Kronstrand and Scott, 2006). The medulla is located in the core of the cortex and is composed of condensed cells that are interspersed with air spaces or aligned continuously (Powell and Rogers, 1997). Hair is synthesized within the hair follicle that is made up of several layers (Kronstrand and Scott, 2006). The outer root sheath surrounds the inner root sheath, which, in turn, encloses the growing hair fiber (Kronstrand and Scott, 2006). At the base of the follicle is the hair bulb, where the germinal matrix and dermal papilla are located. Within the germinal matrix, mitosis takes places, which produces cells that travel upward to form the body of the hair fiber (Patton and Thibodeau, 2016). The dermal papilla contains specialized mesenchymal cells that are involved in the formation, growth and cycling of hair
(Lin et al., 2008). At the apex of the dermal papilla, melanin, which is the pigment that causes colour in hair, is synthesized by melanocytes within melanosomes that are subsequently transported to cells that originate from the hair bulb (Kronstrand and Scott, 2006). Hair growth rate can vary considerably (Kronstrand and Scott, 2006); however, in a study that examined hair growth rate in the general population, 82% of individuals grew between 0.32 and 0.46 mm of hair per day (Pötsch, 1996). The rate of hair growth ranged between 0.07 and 0.78 mm per day (Pötsch, 1996).

Hair growth in utero begins during the 10th week of gestation with the emergence of hair follicles (Furdon and Clark, 2003; Hollbrook, 1998). Cell differentiation produces various components of the hair follicle, such as the dermal papilla (Paus and Cotsarelis, 1999). There are three main stages of hair production: the anagen phase, the catagen phase and the telogen phase. The anagen phase involves active hair production and begins at the 15th week of gestation (Gareri and Koren, 2010). By the 18-20th week the entire scalp is covered with anagen-phase follicles (Gareri and Koren, 2010). The catagen phase involves the degeneration of the lower follicle leading to the full keratinization of hair, also known as club hair (Furdon and Clark, 2003; Paus and Cotsarelis, 1999). This marks the start of the telogen phase, also known as the “resting phase”, which involves loss of the produced hair fibers (Gareri and Koren, 2010). The catagen/telogen phase takes place between the 24-28th weeks of gestation; therefore, neonatal hair present at birth reflects drug exposure after the 28th week of gestation (Hollbrook, 1998; Paus and Cotsarelis, 1999; Gareri and Koren, 2010). The progression of fetal hair growth is illustrated in Figure 7. Future studies should seek to confirm the exact detection time window for neonatal hair analysis. Neonatal hair is available for collection up to approximately 3 months after birth (Gareri and Koren, 2010).
2.7.2. Mechanisms and Factors Associated with the Incorporation of Xenobiotics into Fetal Hair

Xenobiotics are incorporated into fetal hair through two main mechanisms. Substances that are present in the fetal circulation are carried by the capillary blood supply to the hair follicle and deposited into the growing hair shaft (Bailey et al., 1997). Alternatively, drugs may also be directly deposited from circulating amniotic fluid (Bailey et al., 1997). In

Figure 7. Progression of fetal hair growth  
(Reproduced with permission from Furdon and Clark, 2003)
order for a xenobiotic to be incorporated into the matrix cells of growing hair fibers, it has to have the capacity to cross a lipid membrane; therefore, it must be lipid soluble (Kronstrand and Scott, 2006). Furthermore, protein-bound xenobiotics cannot cross the membrane and must be unbound to be able to move across (Kronstrand and Scott, 2006). The pKa of the compound also plays a role in dictating its propensity to be incorporated into hair. Whereas the pH of plasma is 7.3, the pH of melanocytes and keratinocytes (synthesize keratin) is between 3 and 6 (Robbins, 1994). This means that basic compounds are more like to accumulate in hair relative to acidic compounds, because they become protonated upon entering the cytosol of the hair cell, which traps them and prevents them from crossing back to plasma (Kronstrand and Scott, 2006). Once inside the cytosol, xenobiotics can bind to structures within the cell, causing a reduction in the cytosolic concentration (Kronstrand and Scott, 2006). This can function to enhance transport into the hair (Kronstrand and Scott, 2006).

2.7.3. Dose-Response Relationship

A variety of studies have been conducted to determine whether there is a relationship between maternal consumption of xenobiotics during gestation and levels of xenobiotics measured in the hair of offspring. Forman et al. studied the dose-response relationship between cocaine consumption and benzoylecgonine (BZ), and cocaine accumulation in the hair of guinea pig pups exposed to cocaine in utero (1992). At doses of 10, 15 and 20 mg/kg/day, there was a strong linear correlation between cocaine consumption and concentration of BZ found in the hair of pups. Similarly, at doses of 15 and 20 mg/kg/day, there was a significant linear correlation between cocaine consumption and accumulation of cocaine hair. Goodwin et al. assessed the dose-response relationship between maternal dose of buprenorphine and concentrations of buprenorphine and norbuprenorphine in neonatal hair (2007). Due to the small sample size (n=4) a quantitative relationship could not be established; however, it was found that all neonatal hair samples tested positive for the presence of buprenorphine and norbuprenorphine among mothers who were on buprenorphine maintenance during third trimester. Results also revealed a positive linear
correlation between buprenorphine concentration in unwashed maternal hair and neonatal hair.

2.7.4. Establishing Baseline Levels of FAEE in Neonatal Hair

Low levels of ethanol are naturally produced endogenously irrespective of alcohol consumption via normal metabolic processes (Corry, 1978; Boumba et al., 2008; Krebs and Perkins, 1970), such as anaerobic metabolism of carbohydrates (Edwards and Parrett, 2002; Lifschitz et al., 1990; Wolin et al., 1998). This leads to the formation of FAEE that are naturally found throughout the body, including meconium and hair. In order to differentiate between alcohol-exposed and non-exposed neonates, baseline or reference levels of FAEE need to be established. Once FAEE concentrations are determined in both groups, a cut-off value can be calculated which serves to distinguish between exposed and non-exposed neonates. Although several studies have published associations between individual FAEE species and maternal alcohol intake, it is more reliable to quantify the total concentration of multiple common FAEE when assessing alcohol exposure, in order to account for potential differences in fatty acid profiles among populations (Klein et al., 1999; Chan et al., 2003).

Chan et al. established reference levels of FAEE in meconium by analyzing 183 samples derived from neonates born to nondrinking women (2003). Six samples were also collected from neonates of mothers with a confirmed drinking history. It was found that mean levels of FAEE in meconium were higher in exposed (n=11.08 nmol/g, n=6) compared to non-exposed neonates (1.81 nmol/g, n=73). These mean levels represent a total of 6 FAEE and only 73 of the 183 meconium samples collected from non-exposed neonates could be accurately quantified. A cut-off of 2 nmol/g, which combines the levels of the 4 most abundant FAEE (ethyl palmitate, ethyl linoleate, ethyl oleate and ethyl stearate), was established to differentiate between alcohol-exposed and non-exposed neonates, with a sensitivity of 100% and a specificity of 98.4%.

Caprara et al. conducted a pilot study to determine baseline levels of FAEE in the hair of 62 neonates born to teetotalers or mild social drinking mothers (2005b). The mean total FAEE concentration was 0.32 pmol/mg in neonatal hair. Levels of FAEE were quantifiable in 56
samples and the total concentration consists of 6 FAEE (ethyl laureate, myristate, oleate, stearate, palmitate and palmitoleate). Interestingly, it was found that levels of FAEE in neonatal hair did not differ between infants exposed to mild levels of alcohol and those exposed to none.

From a theoretical standpoint, presence of a bacterial infection in either mother or neonate may be associated with increased production of ethanol due to anaerobic metabolism in certain yeasts and bacteria (Jawetz et al., 1982). Certain metabolic disorders, such as gestational diabetes, may also lead to increased ethanol levels in the body (Chan et al., 2003). It has also been demonstrated that gestational diabetes is linked to changes in fatty acid composition within the placenta, umbilical cord and erythrocytes, which can lead to alterations in the levels of FAEE (Lakin et al., 1998). Other clinical variables, such as maternal diet, gestational age and maternal age may also be associated with levels of FAEE (Chan et al., 2003; Pérez-Camino et al., 2002; Lindinger et al., 1997; Sanjurjo et al., 1993). The extent to which these clinical variables modulate FAEE concentrations is relatively unknown and should be further explored in the context of FAEE analysis to ascertain prenatal alcohol exposure.

2.7.5. Analytical Aspects of FAEE Analysis in Neonatal Hair

It is recommended that hair be collected as close as possible to the scalp from the vertex posterior region of the head, because the hair growth rate does not tend to vary as much as other regions of the head or other parts of the body (Cooper et al., 2012; Cooper, 2015). However, in the case of neonatal hair collection, there may not be sufficient hair in this region. Therefore, hair may need to be collected from various parts of the scalp. Upon collecting the hair, the root end should be identified, since the location of a particular segment relative to the root end affects the interpretation of results (Cooper, 2015). Given that neonatal hair is generally very short, hair is not segmented and all the clippings taken from a neonate are mixed together. Following hair collection, samples are kept in a dark environment in order to avoid deterioration of FAEE from direct sunlight (Moore et al., 2003).

The first step of sample preparation involves washing the hair to remove external contamination from the environment and other substances such as sebum and surface
material that may interfere with the analysis of hair (Cooper et al., 2012; Cooper, 2015). This is accomplished by using a non-polar solvent, such as heptane (Cooper, 2015). After drying the hair, it is cut into 1-3 mm pieces to facilitate the extraction of FAEE by increasing the surface area of hair (Pragst et al., 2001). Prior to hair extraction, deuterated versions of each of the four FAEE are added to hair to serve as the internal standards (IS) (Cooper, 2015). The use of ethyl heptadecanoate as an IS has been reported in several studies; however, its use is discouraged because of its presence in the sebum and hair following alcohol intake (Cooper, 2015). FAEE extraction takes place at room temperature for 15 hours and is accomplished by using a two-phase mixture of 0.5 mL dimethyl sulfoxide (DMSO) and 2 mL of heptane (Pragst et al., 2001; Cooper, 2015). The combination of DMSO and heptane is used for the extraction, since it has been demonstrated to generate the highest extraction yield (Pragst et al., 2001; Cooper, 2015). Moreover, this mixture has not been shown to partake in transesterification and ester hydrolysis reactions, as seen with methanol and other alcohols (Pragst et al., 2001; Cooper, 2015). Following extraction, the mixture is cooled below 0°C to allow the lower DMSO layer to freeze (Cooper, 2015). Once frozen, the upper heptane later is decanted and subsequently evaporated (Cooper, 2015). Given that FAEE are non-polar compounds, they are drawn to and retained in the organic heptane layer.

Hair samples may be analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), which combines high performance liquid chromatography (HPLC) with multiple steps of mass spectrometry (MS) selection. HPLC is a separation technique that involves the use of a high-pressure liquid (mobile phase) to force a sample through a column (stationary phase) that is made up of small particles that can facilitate certain types of separation (Ackley and Caruso, 2003). One of the most commonly used HPLC method in the context of LC-MS analysis is reversed-phase liquid chromatography (RP-LC) (Ackley and Caruso, 2003). RP-LC involves the use of a hydrophobic stationary phase and a polar mobile phase (Ackley and Caruso, 2003). The stationary phase column is typically made up of organic-modified particles (Ackley and Caruso, 2003). Examples of columns that are commonly utilized in RP-LC include C18 (octadecylsilane), C8 (octylsilane) or biphenyl columns (Ackley and Caruso, 2003). The mobile liquid phase is usually an aqueous or a pure or pH-adjusted water-organic mixture, such as water-methanol, water-acetonitrile or water-tetrahydrofuran.
(Ackley and Caruso, 2003). In RP-LC, the first molecules to be eluted from the column are typically hydrophilic, because they are less likely to adsorb to the hydrophobic stationary phase and are more likely to remain in the polar mobile phase (Ackley and Caruso, 2003). Conversely, hydrophobic compounds in the mobile phase are more likely to adsorb to the stationary phase (Ackley and Caruso, 2003). The elution of hydrophobic molecules may be accomplished by increasing the concentration of the organic or non-polar solvent in the mobile phase, so that the molecules gravitate toward the mobile phase rather than adsorb to the column (Ackley and Caruso, 2003). Since FAEE are hydrophobic compounds, the analytes of interest initially adsorb to the stationary phase, followed by their elution over time as the concentration of the non-polar solvent increases.

MS is used to identify and quantify molecules in a simple or complex mixture through the generation and separation of ions according to their mass-to-charge (m/z) ratio (Gross, 2011a). All mass spectrometers are composed of an ion source, mass analyzer and ion detector (Gross, 2011a). Tandem MS involves two stages of selection or m/z analysis (Gross, 2011b). Tandem MS can be performed in space and in time (Gross, 2011b). Whereas tandem-in-time involves multiple separation steps taking place in the same place over time, tandem-in-space consists of separation steps take place in distinct but connected locations (Gross, 2011b). When using tandem in space, there are several different modes or scan experiments that can be used, such as precursor ion scan, product ion scan and selected reaction monitoring (SRM) (Gross, 2011b). In the context of FAEE analysis in hair of neonates exposed to little or no alcohol during pregnancy, a highly specific and sensitive analytical approach is required. This is required because hair is a heterogeneous matrix composed of many different types of molecules. Furthermore, levels of FAEE in neonatal hair are expected to be relatively low. Employing the use of SRM can assist in achieving enhanced sensitivity and specificity (Kinter and Kinter, 2013). SRM involves selection of a specific precursor ion, followed by selection and detection of a specific fragment ion that was derived from the original precursor ion (Figure 8). In the first stage, the molecules of the injected sample are ionized and separated according to their m/z ratio (Kinter and Kinter, 2013). This is followed by selection of precursor ions that undergo fragmentation (Kinter and Kinter, 2013). The second stage involves separating the resulting fragment ions by their m/z ratio and detection (Kinter
and Kinter, 2013). The precursor and fragment ion-pair is referred to as a transition. Multiple reaction monitoring (MRM) involves the acquisition of multiple SRM transitions over the course of one experiment (Kinter and Kinter, 2013). MRM is an efficient approach to quantifying multiple FAEE analytes in neonatal hair.

Figure 8. Selected reaction monitoring technique
“Molecular ions of a specific analyte are selected in Q1 and fragmented in Q2. ESI, electrospray ionization. Molecular ions of one or several contaminants are isolated and fragmented together. A specific fragment ion from the target analyte (transition) is selected in Q3 and guided to the detector.”
(Reproduced with permission from Picotti and Aebersold, 2012)

2.7.6. Validation of an Analytical Method

After establishing a new method, it is necessary to determine the reliability of the method and its parameters (FDA, 2015). In order to accurately quantify samples, the sensitivity of the method needs to be established. This involves determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD is the lowest amount of analyte that can be detected in a sample, but not necessarily quantified (Armbruster and Pry, 2008). The LOQ is the lowest amount of analyte that can be reliably quantified in a sample and the point at which some pre-specifed criteria for bias and imprecision are achieved under experimental conditions (Armbruster and Pry, 2008). The LOQ can be either equivalent to the LOD or higher. The linearity of the method, or the ability of the assay to elicit test results that are directly proportional to the concentration of the analyte, within a particular concentration range, should also be established (ICH Expert Working Group, 2005). In ascertaining the
linearity of the assay, the upper limit of quantification can be determined, or the highest concentration of an analyte that can be reliably measured in a sample (ICH Expert Working Group, 2005).

In the context of sample analysis, the extent to which the matrix or its impurities confound the quantitation of the analyte needs to be established. This involves determining the analytical specificity, which refers to the ability of the method to measure the analyte of interest rather than other substances within the sample (ICH Expert Working Group, 2005). The accuracy and precision should also be established. The accuracy of an assay is how close a measured value of the analyte is to the actual value (ICH Expert Working Group, 2005). Precision is how close the measured analyte values are to each other under the same experimental conditions (ICH Expert Working Group, 2005). A measurement system can be accurate but not precise, precise but not accurate, neither, or both. For example, if an experiment contains a systematic error, then increasing the sample size generally increases precision but does not improve accuracy. The result would be a consistent yet inaccurate string of results from the flawed experiment. Eliminating the systematic error improves accuracy but does not change precision.

In order to determine the repeatability of the assay, the intra- and inter-day variability should be ascertained, which assesses the degree of imprecision within a day and over several days of sample analysis, respectively (VICH Expert Working Group, 2009).

Other method parameters that should be determined include extraction efficiency, matrix effect and carryover. An assay’s extraction efficiency is the detector response obtained from an analyte that is added to and recovered from the biological matrix relative to the detector response associated with the true concentration of the analyte present in solvent (FDA, 2001). Although the recovery does not need to be and is rarely 100%, it should be consistent and reproducible (FDA, 2001). The matrix effect refers the degree to which the components of the biological matrix affect the quantitation of the analyte in a given sample (Hall et al., 2012). Compounds within the sample matrix that co-elute with the analytes of interest can interfere with the ionization process leading to ion enhancement or suppression (Hall et al., 2012). Whereas ion enhancement leads to an artificially increased detector
response associated with the analyte of interest, ion suppression is associated with an artificially reduced detector response following analyte elution (Hall et al., 2012). Finally, presence of sample carryover should be determined. Carryover involves the unintended elution or analyte signal of a previously injected sample during the course of analysis of a subsequent sample, which can interfere with analyte quantitation (SWGTOX, 2013). This commonly occurs after the analysis of a sample with a high concentration (SWGTOX, 2013).

Once validation is complete and the assay becomes operational, samples are quantified according to a calibration curve that is run with each batch of samples (FDA, 2001). This involves determining the unknown concentration of a sample using a standard curve made from known concentrations. Quality control (QC) samples are also included in each batch to ensure that adequate analytical performance is maintained throughout sample analysis (FDA, 2001). This is required to ensure that the results are reliable.
Chapter 3
METHODS

3.1. Pharmacogenetics of Opioid Therapy in the Management of Postpartum Pain: A Systematic Review

3.1.1. Search Strategy

A comprehensive search of the literature was conducted on 3–4 June 2015 using the following databases: MEDLINE (articles indexed since 1946 and in-process and other nonindexed citations), EMBASE (since 1980), PubMed, Scopus, Web of Science, CINAHL and Cochrane. The searches for MEDLINE, EMBASE and Scopus were conducted on June 3, while the searches for the remaining databases were completed on June 4. A search strategy was developed to include the search terms: ‘pharmacogenetics’, ‘opioid’ and ‘postpartum’, along with their related terms. The search was conducted by employing the search terms as keywords and exploded subject headings (e.g., Medical Subject Headings). The results of the search were imported into a reference management software (Bookends 12.2.4; Sonny Software, MD, USA).

3.1.2. Inclusion and Exclusion Criteria

To be included in the review, studies had to involve women who were prescribed an opioid for postpartum pain management and who were genotyped for at least one gene relevant to the opioid’s pharmacological pathway. Studies were also required to examine the role of pharmacogenetics in opioid analgesia and/or opioid intake/requirement and/or opioid adverse events in mother and/or infant (via breastmilk) following maternal intake of a prescribed opioid. There was no restriction placed on the language of publication. Studies that examined the pharmacogenetics of an opioid given directly to neonates were excluded. Animal studies, case reports, case series, letters to the editor, review articles and editorials were also excluded. Studies that involved subjects who were addicts or chronic users of opioids were not included. This population was excluded because it is likely to exhibit a distinct sensitivity to opioids, as a result of prior excessive consumption (Morgan & Christie, 2011). Since opioid requirement and analgesia among postpartum patients are the primary
endpoints of most of the studies included in this systematic review, the inclusion of studies containing addicts or chronic users might distort the findings.

3.1.3. Screening for Eligibility

Two reviewers independently screened for eligibility of studies. The initial screen involved excluding duplicate articles arising from overlapping database search results. The following stage involved flagging eligible articles (according to inclusion/exclusion criteria) by scanning abstracts and titles. The final stage involved reviewing the methods and results sections of flagged articles to confirm which studies should be included. At this stage, conference abstracts containing results that were part of a larger published study were excluded to avoid duplicate datasets. Discrepancies at each stage of screening were resolved through discussion between the two reviewers.

3.1.4. Data Extraction and Quality Assessment

Two reviewers independently extracted data and relevant study details using data collection forms and concurrently evaluated the quality of studies. The following information was extracted: year of publication, geographic location, study design, birth years, presence of control group, number of participants, opioid prescribed, polymorphisms under investigation and a brief summary of results on pharmacogenetically-related differences in reported pain, opioid requirement and adverse events in mother and/or infant.

Studies were assessed for quality using the Newcastle–Ottawa Quality Assessment Scale for case–control and cohort studies (Well et al., 2009). The quality of case–control studies was assessed according to selection of cases and controls, comparability of cases and controls and details related to the ascertainment of exposure. The quality of cohort studies was similarly assessed according to selection of exposed and non-exposed cohorts, comparability of cohorts and details related to the ascertainment of outcome. A study can be awarded up to a total of nine stars using this scale. Discrepancies in data extraction and assessment of quality of studies were resolved by discussions between the two reviewers. In the event of an irresolvable disagreement, a third reviewer was available to serve as a tiebreaker. The PRISMA statement was consulted for a checklist of items to include when
reporting a systematic review (Moher et al., 2009).

3.2. Pharmacogenetics of Codeine Pain Relief in the Postpartum Period

3.2.1. Patient Recruitment

The Research Ethics Boards at the University of Western Ontario and St. Michael's Hospital granted ethics approval for this study. Women who delivered via C-section and were prescribed codeine for postpartum pain were approached at St Michael's Hospital. A study coordinator was responsible for explaining the study to prospective participants, answering their questions and obtaining their written and informed consent. Recruitment, follow-up and data collection took place between 1 December 2009 and 30 November 2011 at St Michael's Hospital in Toronto, Canada.

3.2.2. Study Design

In order to manage pain, women were prescribed 1–2 tablets of Tylenol #3 (Church & Dwight, Mississauga, Ontario, Canada - 300 mg acetaminophen and 30 mg of codeine) every 4–6 h as needed. Some women were also prescribed naproxen twice daily (500 mg, every 12h for the first 48 h). Participants were provided with a tracking sheet on which they were instructed to record dosing information and report their levels of pain using a VAS (mm). This information was to be written down 1 h following each dose of codeine for the entire course of medication use. Clinical data were collected, including maternal weight at delivery, age, gravidity, parity, infant birth weight, type of anesthesia administered during delivery and self-described ethnicity. Participants also provided a saliva sample in order to genotype select polymorphisms of the COMT, ABCB1, CYP2D6, UGT2B7 and OPRM1 genes. The polymorphisms that were selected for genotypic analysis are those that have been most commonly reported as being implicated in pain perception and/or opioid analgesia. Moreover, these genes capture the majority of the codeine pharmacokinetic and pharmacodynamic pathways.

Each saliva sample was marked with a unique barcode and shipped to the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Core Laboratory located in Vancouver,
British Columbia, for analysis using a previously described method (Kelly et al., 2013). Participants were assigned to a particular CYP2D6 phenotypic group according to their genotypic activity score, which was calculated by combining the scores of the individual alleles. Functional CYP2D6 alleles (*1 and *2) were given an activity score of 1. Reduced function alleles (*9, *10, *17, *29, *41) were assigned an activity score of 0.5. Non-functional alleles (*3, *4, *6, *7, *8, *12, *14) were given a score of 0. Whole gene deletions (*5) also received an activity score of 0. In the case of whole gene duplications, an activity score of 1 was assigned to each functional copy of CYP2D6. The wild-type allele (*1) was assigned by default in the event that none of the foregoing alleles were detected. The activity scores of PMs, IMs, EMs, and UMs were 0, 0.5-1, 1.5-2, and >2, respectively.

Haplotypes were constructed using polymorphisms of the COMT and ABCB1 genes. The COMT haplotype (rs4633-rs4818-rs4680) consists of three predicted activities: high (CGG), decreased (TCA) and very low (CCG). The ABCB1 haplotype (rs1128503-rs2032582-1045642) is also made up of three different predicted activities: high (CGC), decreased (TTT or CGT) and rare/undefined activity (frequency <5% haplotype).

Both the length of codeine therapy and the number of consecutive days of codeine use varied among subjects. Participants also differed with respect to the date of initiation of codeine therapy following delivery. In order to control for these differences, this study focused on subjects taking codeine on days 1 (same calendar day as the C-section) and 2, since the majority of subjects took codeine for at least the first two calendar days following C-section. Mean pain score (mm), mean dose (mg kg\(^{-1}\)) and cumulative dose (mg kg\(^{-1}\)) information was collected and averaged over days 1 and 2. Pain scores reported using the VAS were measured using a ruler (mm). Dose was standardized to maternal weight to account for differences in participant size, which may influence response to codeine intake. Among the 238 women included in the original cohort study (Kelly et al., 2013), 98 women were identified who met the following criteria: completed their tracking sheet, underwent successful genotyping and took codeine for the first two days following C-section. Women who did not take Tylenol #3 (Church & Dwight) for the first two calendar days following delivery, who were not genotyped and who did not complete the tracking sheet were
3.2.3. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Macintosh, Version 20.0. (IBM Corp., Armonk, NY, USA). In order to establish differences in mean pain score, mean dose and cumulative dose, a one-way analysis of variance was conducted using Tukey’s honest significant difference posthoc test for parametric data with three or more groups of the independent variable. If the assumption of homogeneity of variances was violated, results from the Welch analysis of variance and Games-Howell post-hoc test were obtained. The Kruskal-Wallis test was used as the non-parametric alternative. An independent-samples t-test was conducted for parametric data with independent variables containing two groups and the Mann-Whitney U test was used as the non-parametric alternative. Normality was assessed using Shapiro-Wilk’s test. The non-parametric result was reported if there were deviations from normality. Boxplots were used to detect the presence of outliers. If an outlier was detected, sensitivity analysis was performed whereby the parametric and non-parametric tests were run with the outlier included in all statistical analyses. If both results led to the same conclusion, the outlier was kept in and parametric results were reported, otherwise the non-parametric results were reported. Simple linear regressions were conducted to determine which of the continuous clinical variables significantly predicted average pain score, average dose and mean cumulative dose. Statistically significant findings were incorporated into a multiple linear regression model with stepwise selection. Approximately, half of the participants were also prescribed naproxen, which may confound interpretation of study outcomes. Therefore, this variable was included in all multiple linear regression analyses. Missing values were addressed by excluding cases pairwise.

3.3. Validating the Use of an LC-MS/MS Method in Hair

3.3.1. Blank Child Hair

The Research Ethics Boards at St. Michael’s Hospital and the Hospital for Sick Children (Toronto, Ontario) approved this study. Child hair was used to validate the LC-MS/MS method and for calibrator and QC samples, since it was difficult to acquire large enough quantities of
neonatal hair. The pool of child hair was derived from seven children, aged 18 months to 9 years. The hair was determined to be blank according to gas chromatography – mass spectrometry (GC-MS) analysis following previously published methods (Auwärter et al., 2001; Pragst et al., 2001). It should be noted that child hair is not truly “blank”, since there are naturally occurring levels of FAEE present in the hair. However, they are blank in the sense that the levels are distinguishingly lower than the levels present in alcohol-exposed individual (according to GC-MS analysis). Two different individual pools of hair were created for calibration and QC purposes, respectively. These pools consisted of hair from all seven sources, but in different proportions.

3.3.2. Reagents

Since the Society of Hair Testing 2014 Guidelines recommended the analysis of ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate to determine chronic excessive alcohol consumption in adult hair, these four analytes were chosen to be analyzed in child/neonatal hair (SoHT, 2014). FAEE standards (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) were purchased from Sigma-Aldrich (St. Louis, Missouri). Deuterated FAEE (IS) (D$_5$-ethyl myristate, D$_5$-ethyl palmitate, D$_5$-ethyl oleate, D$_5$-ethyl stearate) were acquired from Toronto Research Chemicals (Toronto, Ontario). HPLC grade heptane and ReagentPlus grade DMSO were from Sigma-Aldrich (St. Louis, Missouri). Optima LC-MS grade methanol and formic acid were purchased from Fisher Scientific (Fair Lawn, New Jersey). Water was purified with a Milli-Q integral water purification system from EMD Millipore Corporation (Darmstadt, Germany).

3.3.3. Instrumentation

FAEE analysis was conducted on a Shimadzu LC-30AD UFLC (Columbia, Maryland) interfaced to an AB Sciex Qtrap$^\text{TM}$ 5500 mass spectrometer with a Turbo V$^\text{TM}$ Ion Source (AB Sciex, Redwood City, California). The LC system consists of an LC-30AD Nexera pump, a SIL-30AC Nexera autosampler, and a CTO-20AC Prominence column oven from Shimadzu Corporation (Columbia, Maryland). LC-MS/MS Analyst 1.6 software (AB Sciex, Redwood City, California) was used to acquire and analyze the data.
3.3.4. Preparation of Standard Solutions

For each of the four FAEE and the four deuterated versions, individual 2 mg/mL stock solutions were prepared in heptane. Separate FAEE and d$_5$-FAEE mix stock solutions were prepared at a concentration of 10 μg/mL. Working calibrator solutions (1.0, 0.1, 0.01, and 0.001 μg/mL) were prepared from the FAEE mix stock solution. A separate set of QC working solutions (1.0 and 0.1 μg/mL) was prepared from the FAEE mix stock solution. The same d$_5$-FAEE working solution (0.1 μg/mL) was used for calibrator, QC and neonatal hair samples. All stock, calibrator and QC solutions were prepared in heptane and stored in 4 mL amber glass vials at -20°C. Prior to use, standard solutions were brought to room temperature for 10 minutes and vortexed for 1 minute.

3.3.5. Sample Preparation and FAEE Extraction Procedure

Sample preparation and FAEE extraction is based on previously published methods (Auwärter et al., 2001; Pragst et al., 2001). All neonatal hair samples underwent a washing procedure, which involved placing hair in 50 mL beakers and immersing samples in 30 mL of heptane for 30 minutes. A spatula was used to stir the submerged samples every 10 minutes. After 30 minutes, the heptane was decanted and the hair samples were left to dry for approximately 30 minutes. The dry hair samples were then placed in 4 mL amber glass vials, cut into 1-3 mm long pieces, and 20 ± 0.5 mg of hair from each sample was transferred to a new glass vial.

Prior to extraction, all samples received 300 pg/mg of d$_5$-FAEE mix IS solution. Calibrator and QC samples were spiked with their respective FAEE mix standard solution. The extraction procedure involved adding 500 µl of DMSO and 2 mL of heptane. Samples were subsequently capped and placed on a shaker at room temperature for 15 hours at 975 rpm. This was followed by placing hair samples in a cold room set at 4°C for 1 hour to allow the DMSO to solidify. The remaining liquid heptane layer was then dispensed into a disposable glass culture tube and evaporated at 37.5°C under nitrogen flow at 10 psi. Once the tubes were completely dry, the samples were reconstituted in 50 µl of HPLC starting mobile phase buffer solution. This consisted of 80% of a 0.1% formic acid in methanol (v/v) solution (B) and
20% of a 0.1% formic acid in water (v/v) solution (A). Samples were subsequently vortexed for 10 seconds, and then dispensed into 50 µl glass inserts. These glass inserts were placed into 1.8 mL amber glass vials and capped. Prior to loading samples onto the LC-MS/MS analysis, samples were vortexed for 5 seconds. During analysis, 10 µl of each sample was injected.

3.3.6. LC-MS/MS

Chromatography was performed using a Kinetex™ 2.6 µm Biphenyl 100 Å 50 x 3.0 mm column (Phenomenex, Torrance, California) fitted with a SecurityGuard™ ULTRA cartridges for UHPLC biphenyl 3.00 mm ID columns (Phenomenex, Torrance, California). The total run time for each sample was approximately 6 minutes and the flow was 0.4 mL/min. The autosampler was set at 4°C and the column oven temperature was maintained at 40°C. To facilitate the separation of FAEE analytes, gradient elution began with 82%B and increased to 90%B over 50 seconds, which was held for 1 minute and 10 seconds. This was followed by an increase to 100%B over 10 seconds that was maintained for 50 seconds. Finally, there was a decrease to 82%B over 10 seconds, which was held for 2 minutes and 50 seconds.

Quantifier and qualifier transition ions were derived from three published studies that conducted FAEE analysis in meconium using LC-MS/MS (Pichini et al., 2008; Kwak et al., 2010; Himes et al., 2014). MS was achieved in positive electrospray ionization mode. FAEE source parameters were optimized via a T-connector infusion of 100 ng/mL FAEE and d5-FAEE mix at a rate of 10 µL/min in start conditions, whereas compound specific MS/MS (tandem mass spectrometry) parameters were optimized via direct MS infusion of 100 ng/mL for each analyte at a rate of 10 µL/min in initial mobile phase conditions. MRM was employed for all analytes with a dwell time of 100 msec.

3.3.7. Validation

Following Clinical and Laboratory Standards Institute (CLSI) guidelines, a number of validation parameters were examined, including sensitivity, specificity, linearity, accuracy, precision, inter- and intra-day variability, carryover, methods comparison and analysis of external quality control (EQC) samples (CLSI, 1997). As mentioned above, child hair was used to conduct the validation experiments. Given that it contains naturally occurring levels of
FAEE, the area ratio of the “blank” or non-spiked sample was subtracted from each point on the calibration curve prior to quantifying any samples that were not derived from the pool of hair used to run the calibration curves. This was required to generate inter and intra-day variability data, to quantify all QC samples, and to quantify samples analyzed using LC-MS/MS in the comparison of methods (GC-MS vs. LC-MS/MS).

*Sensitivity, Linearity and Specificity*

In order to establish the sensitivity of the method, the LOD and LOQ were determined. A range of low concentrations between 1 and 30 pg/mg of FAEE mix were prepared and analyzed in triplicates. The area counts were recorded and the FAEE to d$_5$-FAEE area ratios were calculated and plotted as function of FAEE concentration. At least six concentrations (including the non-zero concentration in a “blank” child hair sample) were included in the calibration curves, which were fitted and analyzed. The LOD and LOQ were determined by using the standard deviation of the response and the slope (ICH, 1996). The LOD was calculated by multiplying the standard error of the y-intercept by three divided by the slope, and the LOQ was calculated by multiplying the standard error of the y-intercept by ten divided by the slope. The final LOD and LOQ for each FAEE were determined by calculating the mean LOD and LOQ derived from the three calibration curves.

To determine linearity, a range of concentrations between 5 and 4000 pg/mg of FAEE mix were spiked into child hair in triplicates. The area counts were recorded and the FAEE to d$_5$-FAEE area ratios were calculated and plotted as function of FAEE concentration. A minimum of 10 concentrations (including the non-zero concentration in a “blank” child hair sample) was included in the calibration curves. The slopes and regression line coefficients were determined for all calibration curves. For each of the four FAEE, linearity was verified by determining the percentage coefficient of variance (%CV) for the slopes and regression line coefficients derived from the triplicate experiments (ICH, 1996). The *apriori* acceptance criteria for linearity were an overall %CV of ≤5 and ≤15% for regression line coefficients and slopes, respectively.
Specificity was assessed by spiking child hair with 300 pg/mg of FAEE mix and 300 pg/mg of d₅-FAEE mix in triplicate, into the separated heptane layer after hair was extracted. The same process was repeated, but directly into mobile phase (without hair). The FAEE to d₅-FAEE area ratios were calculated and the retention times of all analytes were recorded for the triplicates analyzed with and without hair. The overall mean, standard deviation and %CV, which combined the results with and without hair, were calculated. The apriori acceptance criteria for retention times and area ratios were ≤2.5% and ≤20% CV, respectively.

**Accuracy, Precision and Carryover**

Accuracy and precision were evaluated by analyzing three replicates of child hair spiked at 70 and 300 pg/mg of FAEE mix. To calculate accuracy, the percent recovery was calculated for each analyte at both concentrations by dividing the calculated mean analyte concentration (based on the standard curve) by the expected concentration, which was expressed as a percentage. The apriori acceptance criterion for FAEE recovery was a %CV of <10%.

To assess precision, the %CV of the area count ratios were calculated for each analyte at both concentrations. The acceptance criterion for the area ratios were a %CV of ≤20%. Intra-day variability was assessed by analyzing five replicates of child hair each spiked at two levels (70 and 300 pg/mg of FAEE mix). The %CV for the calculated concentrations (based on the standard curve) within the same day was determined. Inter-day variability was assessed by repeating the same experiment described for intra-day variability on three separate days. Using the intra-day variability data, together with the data acquired over the three additional days, the %CV for the calculated concentrations over four days was obtained.

Carryover was investigated by assessing the area counts present in mobile phase for each of the four FAEE following LC-MS/MS analysis of a sample spiked with a high concentration (4000 and 12,000 pg/mg of FAEE mix). This was assessed on two separate days.
To assess the external validity of the results, adult hair samples containing pre-specified amounts of FAEE were acquired from MEDICHEM Diagnostica (Steinenbronn, Germany) and tested using the LC-MS/MS method. To evaluate the accuracy of the results, the percent recovery was determined for each of the four FAEE by dividing the calculated concentrations by the expected concentration and expressing the result as a percentage.

To ensure the reliability of the results derived via LC-MS/MS, an experiment was performed to compare the results derived via GC-MS analysis (Pragst et al., 2001) to those derived via LC-MS/MS analysis. Five replicates of child hair at six different concentrations (70, 300, 500, 1000, 1500 and 2000 pg/mg) of FAEE mix were analyzed using both GC-MS analysis and LC-MS/MS analysis. To assess the comparability of the methods, a correlation coefficient was calculated for each of the FAEE based on the calculated concentrations derived from the two sets of analyses.

3.3.8. Extraction Efficiency and Matrix Effect

Extraction efficiency and matrix effect were established via post-extraction addition (SWGTOX, 2013; Matuszewski et al., 2003). Three sets of samples were prepared. The first set consisted of four child hair samples that were spiked with 300 pg/mg of d5-FAEE mix, which underwent the typical extraction and LC-MS/MS analysis procedure. The second set consisted of four child hair samples that were spiked with 300 pg/mg of d5-FAEE mix into the isolated heptane layer after extraction had taken place. The third set involved spiking 300 pg/mg of d5-FAEE mix directly into mobile phase, avoiding the extraction procedure altogether. In order to assess extraction efficiency, the mean IS peak areas of set 1 were divided by the mean IS peak areas of set 2 for each of the four deuterated FAEE. The matrix effect was established by dividing the mean IS peak areas of set 2 by the mean IS peak areas of set 3 for each of the four deuterated FAEE. All results were converted to a percentage. For matrix effect, the resulting percentages were subtracted from 100. Ion enhancement and suppression were associated with positive and negative values, respectively. The mean peak areas of the non-deuterated analytes of interest were not used to evaluate extraction efficiency and matrix
effect, because child hair contains naturally occurring levels of FAEE, which obscure the measurement of these parameters.

3.4. Establishing Baseline Levels of FAEE in Neonatal Hair

3.4.1. Subject Recruitment

The Research Ethics Boards at St. Michael’s Hospital and the Hospital for Sick Children (Toronto, Ontario) approved this study. Women who had a pregnancy resulting in a live birth and who did not have a documented history of alcohol or substance use were approached.

This study aimed to analyze baseline levels of FAEE in 200 neonatal hair samples. Recruitment took place at the Postpartum Unit of St. Michael’s Hospital in Toronto, Ontario. Women who signed informed consent forms, and reported abstinence from alcohol or low levels of use (less than or equal to one drink per week) during the last two trimesters of pregnancy were deemed eligible for the study. Neonatal hair samples were collected within several days of birth. Hair samples were obtained by cutting at least 20 mg of hair as close to the scalp as possible. Time and date of collection were recorded, and hair samples were placed in small envelopes for transport. Prior to hair analysis, samples were stored in the dark in order to minimize FAEE degradation (Moore et al., 2003). Along with hair collection, women answered questions to a page-long questionnaire. The following information was collected: frequency of alcohol beverage consumption in the last two trimesters of pregnancy, maternal age, maternal infection and use of antibiotics around time of delivery, maternal medical conditions (such as diabetes), pregnancy complications around time of birth, date and time of birth, gestational age at birth, neonatal complications, neonatal antibiotics around time of delivery and date of first bath.

A study coordinator explained the study to potential subjects, answered questions, obtained written and informed consent, cut and collected the neonatal hair samples and filled out questionnaires based on self-reported information derived from the subjects or their health-care providers.
3.4.2. LC-MS/MS analysis of neonatal hair samples

Prior to analyzing the neonatal hair samples, a Levey-Jennings QC chart was constructed and populated using the data derived from the inter-day variability analyses. The neonatal hair samples were analyzed in five separate batches. Each batch was run with a calibration curve and three QC samples. The range of the calibration curve was from 0 to 500 pg/mg with at least six FAEE concentrations included. The low and high QC samples were 70 and 300 pg/mg, respectively. Prior to quantifying the neonatal hair samples, the area ratio of the “blank” or non-spiked sample was subtracted from each point on the calibration curve. Concentrations of FAEE analytes below their respective LOD or LOQ were considered to be unquantifiable and were not included in the assessment of baseline FAEE levels and statistical analyses.

3.4.3. Statistical Analysis

To investigate whether there is a relationship between the levels of FAEE in neonatal hair and the clinical characteristics obtained from the questionnaires, statistical analyses were conducted. IBM SPSS Statistics for Macintosh Version 20.0. (IBM Corporation, Armonk, New York) was used to perform statistical tests. Normality of the data was assessed using normal Q-Q plots and outliers were identified using boxplots. If data was not normally distributed, the non-parametric result was reported. To establish the association between a continuous independent and dependent variable, Pearson’s correlation was performed and Spearman’s correlation was used as the non-parametric alternative. An independent-samples t-test was conducted if the independent variable contained two groups and the dependent variable was continuous. The non-parametric alternative was the Mann-Whitney U test. For parametric data, a one-way ANOVA was performed if the dependent variable was continuous and the independent variable contained three or more groups. The Kruskal-Wallis test was used in the event that the data was non-parametric. To control for familywise error rate, the Bonferroni correction was applied.
Chapter 4
RESULTS

4.1. Pharmacogenetics of Opioid Therapy in the Management of Postpartum Pain: A Systematic Review

4.1.1. Study Selection

A total of 2082 articles were retrieved following a comprehensive search of the seven databases listed above. Following removal of 970 duplicate articles, 1112 articles were screened according to the inclusion/exclusion criteria based on their title and abstract. This study selection strategy resulted in the exclusion 1077 articles. The subsequent review of the methods and results section of the 35 remaining articles led to the exclusion of an additional 18 articles. Hence, after excluding articles that did not meet the inclusion criteria and duplicate datasets, 17 articles remained. A summary of the study selection process is provided by Figure 9. The 17 studies that were included involved a variety of study designs, opioids prescribed for postpartum pain, polymorphisms under investigation and end points. One of the included articles was a conference abstract (Ciszkowski et al., 2010) and another article was published in Chinese (Xu et al., 2015), which was translated into English with the assistance of an online translation tool. Pertinent study details and a brief synthesis of findings related to the studies are summarized according to opioid treatment in Tables 4–7.
4.1.2. Quality of Studies

Among the published codeine studies, the majority are of low quality, scoring four stars or fewer (Ciszkowski et al., 2010; Madadi et al., 2009; VanderVaat et al., 2011a; Kelly et al., 2013; Baber et al., 2015). One study was awarded five stars, which suggests moderate quality (Sistonen et al., 2012). The cohort studies on morphine from Singapore are of low quality (≤four stars) (Sia et al., 2008; Tan et al., 2009; Sia et al., 2010), while the study from Taiwan is of moderate quality (five stars) (Tsai et al., 2010). All of the cohort studies on hydrocodone are of low quality (≤four stars) (Wong et al., 2010; Boswell et al., 2013; Stauble et al., 2014). The two studies on sufentanil are of moderate quality (six stars) (De Capraris et al., 2011) and low quality (four stars) (Xu et al., 2015). The studies on oxycodone (Lam et al., 2013) and fentanyl (Zhang et al., 2013) are also of low quality (≤four stars). A comprehensive view of the quality assessment of individual studies is available in Table 3.
4.1.3. Codeine

In Canada, Koren and colleagues conducted work related to codeine pharmacogenetics in the postpartum period. Among the six studies published between 2009 and 2015, two nested cohort studies investigated the role of codeine pharmacogenetics in women recovering from C-section. VanderVaart et al. conducted a pilot study in 45 women to examine the role of CYP2D6 (2011a). Two PMs reported no analgesia despite higher codeine consumption compared with the other genotypic groups. Moreover, two of the three UMs reported immediate pain relief following small doses, but due to dizziness and constipation, preferred alternative therapies. Among a nested cohort of 98 women, Baber et al. found significant differences in mean dose consumption between the genotypic groups of OPRM1 A118G and UGT2B7 C802T genes (2015). Individuals carrying the AG genotype and CC genotype consumed more than those carrying the AA and TT genotype, respectively (Baber et al., 2015). These variants significantly predicted codeine consumption in the cohort overall (p < 0.001) and among Caucasians (p = 0.001). The PMs consumed more codeine than the other
phenotypes, however, this did not reach significance.

The relationship between pharmacogenetics and adverse events were explored in two cohort studies and two case–control studies. Kelly et al. did not find any significant association between any of the genes analyzed and maternal adverse events or infant sedation in a cohort of 238 women following C-section (2013). Moreover, codeine intake did not differ among the CYP2D6 phenotypes. In a conference abstract, Ciszkowski et al. interviewed 394 breastfeeding mothers receiving acetaminophen alone or acetaminophen in combination with codeine (2010). Four mothers of infants with CNS depression were CYP2D6 UMs. It is unclear whether there were any formal statistics conducted to assess the relationship between CYP2D6 phenotype and CNS depression in infants, as limited information was available in the abstract. Since Ciszkowski et al. (2010) was a conference abstract, Lam et al. was consulted for additional study details, as it contains the published dataset (2012).

In a case–control study examining the genetic risk factors associated with CNS depression in 72 breastfeeding infants, Madadi et al. found that two (11.8%) mothers of symptomatic infants were CYP2D6 UMs in combination with UGT2B7 TT genotype (for the UGT2B7 C802T variant) (2009). It was demonstrated that the mothers of symptomatic infants were more than eight-times more likely to have the combined genotype than the average expected Western European population frequency of 1.4% (odds ratio [OR]: 8.4; 95% CI: 4.7–47; p ≤ 0.001). In the case–control study by Sistonen et al., CNS depression in mothers and infants was associated with maternal CYP2D6 phenotypic status and TT genotype for the ABCB1 G2677AT variant among 111 mother–infant pairs (2012). After adjusting for maternal age and dose, the associations remained significant. The maternal high-risk genotype was defined as the following: CYP2D6 UM + ABCB1 2677 no T/T or CYP2D6 EM + ABCB1 2677T/T or CYP2D6 UM + ABCB1 2677T/T. A multilocus risk score combining the effects of the associated maternal risk genotypes was more significantly associated with both infant (OR: 2.68; 95% CI: 1.61–4.48; Ptrend = 0.0002) and maternal CNS depression (OR: 2.74;95% CI: 1.55–4.84; Ptrend = 0.0005) as compared with each genotype alone. Furthermore, the risk of codeine-induced CNS depression was increased sevenfold (OR: 7.10; 95% CI: 2.65–18.98; P_{trend}
= 9.4 x 10^{-5}) in the mother–infant pairs classified as being at high genetic risk, as compared with the low-risk group.

It is important to keep in mind that the two case–control studies (Madadi et al., 2009; Sistonen et al., 2012), which examined adverse events, contain a heterogeneous breastfeeding patient population that received codeine for other indications, including postpartum pain relief.
Table 4. Characteristics and reported outcomes of studies investigating the pharmacogenetics of codeine

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Geographic location</th>
<th>Study design</th>
<th>Birth years</th>
<th>Study participants</th>
<th>Polymorphisms under investigation</th>
<th>Main outcomes relevant to analgesia</th>
<th>Main outcomes relevant to adverse events</th>
<th>Newcastle-Ottawa Scale Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madadi et al. (2009)*</td>
<td>Canada-wide</td>
<td>Case-control</td>
<td>2004–2007</td>
<td>72–17 symptomatic and 55 asymptomatic infants</td>
<td>CYP2D6, UGT2B7 C802T2</td>
<td>NA</td>
<td>11.8% of mothers with symptomatic infants were CYP2D6 UMs combined with UGT2B7 TT genotype. Mothers of cases are more than 8 times more likely to have the combined genotype</td>
<td>3</td>
</tr>
<tr>
<td>Ciszkowski et al. (2010)*</td>
<td>Canada-wide</td>
<td>Cohort</td>
<td>2004–2008</td>
<td>394–184 acetaminophen and 210 acetaminophen + codeine</td>
<td>CYP2D6</td>
<td>NA</td>
<td>Unclear whether formal statistics were conducted to assess CYP2D6 association with adverse events. Four mothers of babies with CNS depression were CYP2D6 UMs</td>
<td>4</td>
</tr>
<tr>
<td>VanderVaart et al. (2011)</td>
<td>Toronto, Canada</td>
<td>Nested Cohort</td>
<td>2008–2009</td>
<td>45</td>
<td>CYP2D6</td>
<td>No statistically significant results. Two PMs reported no analgesia and consumed more codeine than the other phenotypic groups. Two of 3 UMs reported immediate pain relief</td>
<td>No statistically significant results. Two of 3 UMs reported dizziness and constipation</td>
<td>3</td>
</tr>
<tr>
<td>Sistonen et al. (2012)*</td>
<td>Canada-wide</td>
<td>Case-control</td>
<td>2004–2008</td>
<td>111–26 symptomatic and 85 asymptomatic infants, 21 symptomatic and 90 asymptomatic mothers, 15 symptomatic and 96 asymptomatic mother-infant pairs</td>
<td>COMT rs4633/rs4818/rs4680, ABCB1 G1236T/G2677AT/C3435T/haplootype, CYP2D6, UGT2B7 C802T2/rs62298861, OPRM1 A118G/rs563649</td>
<td>NA</td>
<td>CYP2D6 (UM/EM) and ABCB1 2677T homozygotes are associated with CNS depression in mother and infant. A genetic model combining maternal risk genotype was significantly associated with both infant and maternal CNS depression. None of the other polymorphisms were associated with adverse events</td>
<td>5</td>
</tr>
<tr>
<td>Kelly et al. (2013)</td>
<td>Toronto, Canada</td>
<td>Cohort</td>
<td>2009–2011</td>
<td>238</td>
<td>COMT rs4633/rs4818/rs4680, ABCB1 G1236T/G2677AT/C3435T, CYP2D6, UGT2B7, OPRM1 A118G</td>
<td>Dose consumption did not vary among CYP2D6 phenotypic groups</td>
<td>Polymorphisms were not associated with maternal adverse events or infant sedation</td>
<td>4</td>
</tr>
<tr>
<td>Baber et al. (2015)</td>
<td>Toronto, Canada</td>
<td>Nested Cohort</td>
<td>2009–2011</td>
<td>98</td>
<td>COMT rs4633/rs4818/rs4680/haplootype, ABCB1 G1236T/G2677AT/C3435T/haplootype, CYP2D6, UGT2B7, OPRM1 A118G</td>
<td>UGT2B7 TT consumed a lower mean dose than CC. OPRM1 A6 consumed a higher mean dose than AA. CYP2D6 PM consumed more codeine (not statistically significant). The remaining polymorphisms and haplotypes were not associated with reported pain scores or cumulative dose intake</td>
<td>NA</td>
<td>3</td>
</tr>
</tbody>
</table>

*Proportion of participants were not treated for postpartum pain.
*Conference abstract.
EM: Extensive metabolizer; NA: Not applicable; PM: Poor metabolizer; UM: Ultra-rapid metabolizer.
4.1.4. Morphine

In Singapore, Sia and Tan et al. have conducted the bulk of the work related to morphine pharmacogenetics following C-section. Sia et al. examined the role of OPRM1 A118G in morphine analgesia and adverse events in a cohort of 588 patients (2008). Women with the AA genotype consumed less morphine, reported lower pain scores, had a higher incidence of nausea and reported higher severity scores for nausea than AG and GG individuals. After adjusting for age and payment class, the OPRM1 genotype was found to significantly affect total morphine intake (p < 0.0001), with each additional copy of the G allele increasing total morphine intake by 1.87 mg (CI: 0.95–2.79). Similarly, after adjusting for a variety of demographic parameters, the OPRM1 genotype was found to significantly explain the variation in pain (p = 0.014), with each additional G allele increasing pain scores by 0.51 units on a 0–100 VAS (CI: 0.10–0.91).

Tan et al. explored the influence of two OPRM1 polymorphisms in a cohort of 994 women (2009). There were differences in the genotypic groups of OPRM1 A118G with respect to time-averaged self-rated pain scores (p = 0.024) and total (p = 1.7 x 10^{-5}) and weight-adjusted morphine (p = 6.6 x 10^{-5}) consumption during the first 24-h postoperative period. It was found that 118G homozygotes consumed more morphine (total and weight-adjusted) than 118A carriers. With respect to pain, 118A homozygotes reported a lower time-averaged pain score than 118G carriers. Furthermore, although the 118G allele was associated with a reduced risk of nausea and vomiting in the population overall, statistical significance was lost after stratifying according to ethnicity.

Sia et al. investigated the role of ABCB1 polymorphisms in postpartum analgesia and morphine side effects in a cohort of 620 women (2010). Individuals with the TT genotype for the ABCB1 C3435T variant had the longest mean survival time of wound pain as compared with CT (p = 0.004) and CC (p = 0.014) genotypes. In Taiwan, Tsai et al. examined the role of OPRM1 A118G in pruritus in a cohort of 212 women being treated with morphine for post-cesarean pain (2010). Analyses revealed that individuals homozygous for 118G had a significantly lower incidence of central type pruritus than 118A carriers (p = 0.031). There was a trend toward the GG group having the lowest severity score of pruritus, however, this did
not reach statistical significance.

Table 5. Characteristics and reported outcomes of studies investigating the pharmacogenetics of morphine

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Geographic location</th>
<th>Study design</th>
<th>Birth years</th>
<th>Study participants</th>
<th>Polymorphisms under investigation</th>
<th>Main outcomes relevant to analgesia</th>
<th>Main outcomes relevant to adverse events</th>
<th>Newcastle–Ottawa Scale Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sia et al. (2008)</td>
<td>Singapore</td>
<td>Cohort</td>
<td>2006–2007</td>
<td>588</td>
<td>OPRM1 A118G</td>
<td>AA individuals had a higher incidence of nausea and higher severity scores for nausea than AG and GG individuals</td>
<td>AA individuals had a higher incidence of nausea and higher severity scores for nausea than AG and GG individuals</td>
<td>4</td>
</tr>
<tr>
<td>Tan et al. (2009)</td>
<td>Singapore</td>
<td>Cohort</td>
<td>2005–2007</td>
<td>994</td>
<td>OPRM1 A118G/T172G haplotype</td>
<td>118G homozygotes consumed more morphine than 118A carriers. 118A homozygotes reported a lower pain score than 118G carriers. OPRM1-T172G and haplotype was not associated with analgesia</td>
<td>118G allele was associated with reduced risk of nausea and vomiting. OPRM1-T172G and haplotype was not associated with analgesia</td>
<td>4</td>
</tr>
<tr>
<td>Sia et al. (2010)</td>
<td>Singapore</td>
<td>Cohort</td>
<td>2006–2007</td>
<td>620</td>
<td>ABCB1 C1236T/G2677AT/C5345T</td>
<td>3435 TT carriers had the longest mean survival time of wound pain compared with CT and CC carriers. The other polymorphisms were not associated with total pain scores or morphine intake</td>
<td>No significant findings related to side effects (nausea, pruritus, CNS or respiratory depression)</td>
<td>3</td>
</tr>
<tr>
<td>Tsai et al. (2010)</td>
<td>Taipei City, Taiwan</td>
<td>Cohort</td>
<td>2007–2008</td>
<td>212</td>
<td>OPRM1 A118G</td>
<td>NA</td>
<td>118 GG individuals had a lower incidence of central type pruritus than 118A carriers. There was a trend toward the GG group having lowest severity score</td>
<td>NA: Not applicable.</td>
</tr>
</tbody>
</table>

4.1.5. Hydrocodone

There are three published studies from the USA related to the role of hydrocodone pharmacogenetics in the postpartum period. Wong et al. investigated the role of OPRM1 A118G in a cohort of 103 women (2010). Hydrocodone intake was converted to oral morphine equivalents. It was found that genotype was not associated with VAS scores at rescue, satisfactions scores with respect to analgesia, time to first rescue medication, morphine intake at various time points and over 72 h, or incidence of nausea. However, the incidence of pruritus was lower in AG and GG individuals, compared with AA individuals (p = 0.02).

Similarly, Boswell et al. studied OPRM1 A118G polymorphism in a post-cesarean
cohort of 158 women (2013). The work revealed that pain relief was significantly associated with total hydrocodone dose ($p = 0.01$) and serum hydromorphone concentration ($p = 0.004$) in the AA group, but not in the AG/GG group. Moreover, side effects were significantly lower in the AA group than in the AG/GG group ($p < 0.04$) after adjusting for BMI, pain level and total dose of hydrocodone. Furthermore, it was found that patients with the AG/GG genotype were 2- to 2.5-times more likely to report constipation, dizziness, dry mouth, vomiting and weakness; and five-times more likely to report respiratory depression.

From the same group, Stauble et al. explored the role of $CYP2D6$ in a cohort of 156 post-cesarean patients prescribed hydrocodone (2014). $CYP2D6$ phenotypic status was not associated with pain index scores, side effects (confusion, constipation etc.) or treatment duration. There was a trend toward PMs consuming a greater total dose than UMs, EMs and IMs.

Table 6. Characteristics and reported outcomes of studies investigating the pharmacogenetics of hydrocodone

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Geographic location</th>
<th>Study design</th>
<th>Birth years</th>
<th>Study participants</th>
<th>Polymorphisms under investigation</th>
<th>Main outcomes relevant to analgesia</th>
<th>Main outcomes relevant to adverse events</th>
<th>Newcastle–Ottawa Scale Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong et al. (2010)</td>
<td>Chicago, IL, USA</td>
<td>Cohort</td>
<td>Not stated</td>
<td>103</td>
<td>$OPRM1$ A118G</td>
<td>Genotype not associated with VAS at rescue, satisfaction scores with respect to analgesia, time to first rescue medication, or morphine intake at various time points and over 72 h</td>
<td>Genotype not associated with incidence of nausea. The incidence of pruritus was lower in AG and GG individuals than in AA individuals</td>
<td>3</td>
</tr>
<tr>
<td>Roswell et al. (2013)</td>
<td>Louisville, KY, USA</td>
<td>Cohort</td>
<td>2008–2011</td>
<td>158</td>
<td>$OPRM1$ A118G</td>
<td>Reported pain was significantly associated with total hydrocodone dose and serum hydromorphone concentration in the AA group, but not in the AG/GG group</td>
<td>Side effects were 4 significantly lower in the AA group than in the AG/GG group</td>
<td>4</td>
</tr>
<tr>
<td>Stauble et al. (2014)</td>
<td>Louisville, KY, USA</td>
<td>Cohort</td>
<td>Not stated</td>
<td>156</td>
<td>$CYP2D6$</td>
<td>PMs consumed a greater total dose than the other phenotypic groups (trend only). Phenotypic status was not associated with pain index or treatment duration</td>
<td>Phenotypic status was not associated with side effects</td>
<td>3</td>
</tr>
</tbody>
</table>

*Hydrocodone converted to oral morphine equivalents. VAS: Visual analog scale.

4.1.6. Sufentanil, Oxycodone and Fentanyl

Two studies have examined the role of sufentanil pharmacogenetics among post-cesarean patients with respect to the $OPRM1$ A118G variant. In Italy, De Capraris et al. randomized 41 women to receive analgesia with or without sufentanil (2011). At 6 h
postoperative, heterozygous patients (AG) reported higher pain scores than wild-type patients (AA), but at 24 and 48 h post-operative the opposite was reported. These results were not statistically significant. In China, Xu et al. did not find an association between genotype and reported pain scores at various time points, satisfaction scores with respect to analgesia, total consumption of sufentanil or side effects (nausea and pruritus) (2015).

In Canada, our group investigated the role of various polymorphisms in the incidence of CNS depression in mothers and infants following oxycodone use in 67 breastfeeding mothers. Lam et al. demonstrated that mothers carrying at least one copy of the \textit{ABCB1} 2677T variant had an increased risk of experiencing sedation themselves (OR: 2.25; 95% CI: 1.06–5.28; \(p = 0.03\)) (2013). Among the three CYP2D6 UMs, one reported oxycodone-induced CNS depression in her infant. It should be kept in mind that a proportion of the breastfeeding mothers received oxycodone for indications other than postpartum pain.

Finally, Zhang et al. investigated the role of the \textit{OPRM1} A118G variant in a cohort of 96 post-cesarean subjects taking fentanyl for postoperative pain (2013). It was found that individuals with GG genotype reported higher pain scores at 12 (\(p < 0.05\)) and 24 h (\(p < 0.05\)) postoperatively, and consumed more fentanyl over 48 h than AG and GG individuals (\(p < 0.05\)).
4.2. Pharmacogenetics of Codeine Pain Relief in the Postpartum Period

4.2.1. Participant Inclusion and Clinical Characteristics

In the original study cohort, 268 women were approached and 255 of them consented to participate (Kelly et al., 2013). Among the 255 women who consented to participate, 15 were lost to follow-up and 1 withdrew for personal reasons. The nested cohort in this study consisted of 98 women who were successfully genotyped, completed the tracking sheet and took codeine for at least the first 2 days following C-section. Aside from two participants who were unsuccessfully genotyped for CYP2D6 and UGT2B7, there were no missing data. The average hospital stay for women with a C-section was 3 days. Clinical characteristics of study participants are described in Table 8. Among participants, 53.1% received epidural anesthetic,
45.9% were given spinal anesthetic and 1% were administered both. There was no difference in pain control between subjects who received epidural, spinal and combined spinal and epidural anesthesia. Approximately, half of the participants were also prescribed naproxen (48% prescribed vs 52% not prescribed). There was no difference in the control of pain between women who took and did not take naproxen. Simple linear regression analyses revealed that increasing maternal age is predictive of a higher reported mean pain score (Table 8). There was also a statistically significant difference in mean dose intake between ethnicities. Caucasians (median = 0.78 mg kg$^{-1}$; range = 0.48 mg kg$^{-1}$) consumed a lower mean dose compared to Asians (median = 0.94 mg kg$^{-1}$; range = 0.51 mg kg$^{-1}$) (Table 8). None of the other clinical characteristics (maternal weight, birth weight, parity, gravidity, type of anesthesia and prescription for naproxen) were statistically significant for mean pain score and mean and cumulative dose consumption.

### Table 8. Clinical characteristics of subjects following C-section, n=98

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Mean ± s.d. or raw value and (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pain score (mm)$^a$</td>
<td>23.85 ± 13.11</td>
</tr>
<tr>
<td>Mean dose (mg kg$^{-1}$)$^b$</td>
<td>0.81 ± 0.12</td>
</tr>
<tr>
<td>Cumulative dose (mg kg$^{-1}$)</td>
<td>2.54 ± 1.01</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>33.46 ± 4.53</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>73.90 ± 10.25</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3374.83 ± 509.51</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.32 ± 1.43</td>
</tr>
<tr>
<td>Parity</td>
<td>1.71 ± 0.84</td>
</tr>
<tr>
<td>Epidural anesthetic</td>
<td>52 (53.1)</td>
</tr>
<tr>
<td>Spinal anesthetic</td>
<td>45 (45.9)</td>
</tr>
<tr>
<td>Epidural and spinal anesthetic</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Prescribed naproxen</td>
<td>47 (48)</td>
</tr>
<tr>
<td>Not prescribed naproxen</td>
<td>51 (52)</td>
</tr>
</tbody>
</table>

**Ethnicity**

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>38 (38.8)</td>
</tr>
<tr>
<td>Asian</td>
<td>15 (15.3)</td>
</tr>
<tr>
<td>South Asian</td>
<td>10 (10.2)</td>
</tr>
<tr>
<td>African</td>
<td>12 (12.2)</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9 (9.2)</td>
</tr>
<tr>
<td>Arabic</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (2.0)</td>
</tr>
</tbody>
</table>

$^a$Simple linear regression shows that maternal age predicts mean pain score [$F (1,96)=4.27$, $P=0.041$, adjusted $R^2=0.033$ and predicted mean pain score = $3.87+0.60$ x maternal age]. $^b$Differences in mean dose were seen between ethnic groups ($\chi^2 (8)=16.06$, $P=0.042$). Differences in mean dose intake between Caucasians (median = 0.78 mg kg$^{-1}$; range = 0.48 mg kg$^{-1}$) and Asians (median = 0.94 mg kg$^{-1}$; range = 0.51 mg kg$^{-1}$), adjusted $P=0.048$, but not between other groups.
4.2.2. Pharmacogenetic Analysis

Among polymorphisms related to the codeine pathway, there were differences in mean dose consumption for the *UGT2B7* and *OPRM1* genes. A difference in mean dose intake was observed for the *UGT2B7* C802T variant. Homozygous mutant participants, TT (0.76 ± 0.12 mg kg\(^{-1}\)), consumed a lower mean dose than homozygous wild-type subjects, CC (0.86 ± 0.11 mg kg\(^{-1}\); Table 9; Figure 10). Mean dose intake was also lower for participants with the AA genotype (0.77 ± 0.13 mg kg\(^{-1}\)) of the *OPRM1* A118G variant compared to those with the AG genotype (0.87 ± 0.092 mg kg\(^{-1}\); Table 9; Figure 10). No differences in mean pain score or dose intake were detected among other polymorphisms in the codeine pathway or among the predicted activities of the COMT and ABCB1 haplotypes (Table 9). Variables that were statistically significant were included in a multiple linear regression model to determine patient characteristics that predict mean dose intake. Ethnicity, *UGT2B7* rs7439366, *OPRM1* rs1799971 and prescription for naproxen (to control for confounding) were entered into the model. Stepwise selection revealed that *UGT2B7* rs7439366 (P = 0.002) and *OPRM1* rs1799971 (P < 0.001) predict mean dose intake in this cohort, which together account for 18.4% of the interindividual variability (Table 10). Given that a difference in mean dose intake was observed between Caucasians and Asians—and that the literature suggests these ethnicities differ with respect to their allele frequencies for the *UGT2B7* and *OPRM1* polymorphisms (which was also observed in our study)—multiple linear regression models were constructed to determine if the *UGT2B7* and *OPRM1* polymorphisms play a role in mean dose intake reported by Caucasians and Asians. Multivariate analysis revealed that both *UGT2B7* rs1799971 (P = 0.046) and *OPRM1* rs1799971 (P = 0.002) predict mean codeine intake by Caucasians, which together account for 28% of the variation in the dependent variable (Table 10). Neither polymorphism was predictive of mean dose intake by Asians.
Table 9. Influence of genetic factors on mean pain score (mm), mean dose (mg kg\(^{-1}\)) and cumulative dose (mg kg\(^{-1}\)) intake reported by subjects, n=98

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype or phenotype groups</th>
<th>Genotype or phenotype frequency</th>
<th>Mean pain (mm)</th>
<th>P-value</th>
<th>Mean dose (mg kg(^{-1}))</th>
<th>P-value</th>
<th>Cumulative dose (mg kg(^{-1}))</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMT</td>
<td>rs4633</td>
<td>CC</td>
<td>0.247</td>
<td>25.08 (45.26)</td>
<td>0.487</td>
<td>0.79±0.12</td>
<td>0.336</td>
<td>2.21 (3.72)</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>0.520</td>
<td>23.44 (56.13)</td>
<td>0.81±0.12</td>
<td>2.26 (4.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>0.133</td>
<td>16.63 (49.62)</td>
<td>0.84±0.13</td>
<td>2.57 (3.65)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs4818</td>
<td>CC</td>
<td>0.388</td>
<td>21.82±13.30</td>
<td>0.419</td>
<td>0.81±0.12</td>
<td>0.953</td>
<td>2.39 (3.65)</td>
<td>0.839</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>0.520</td>
<td>25.30±13.25</td>
<td>0.81±0.13</td>
<td>2.26 (4.28)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>rs4680</td>
<td>CC</td>
<td>0.337</td>
<td>25.04±45.01</td>
<td>0.505</td>
<td>0.80±0.13</td>
<td>0.563</td>
<td>2.25 (3.72)</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>0.092</td>
<td>23.03±11.40</td>
<td>0.82±0.11</td>
<td>2.40 (3.15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>0.520</td>
<td>24.13±56.13</td>
<td>0.80±0.12</td>
<td>2.31 (4.17)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
<td>0.143</td>
<td>17.44±49.62</td>
<td>0.84±0.13</td>
<td>2.54 (3.65)</td>
<td></td>
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<tr>
<td>COMT</td>
<td>CGG</td>
<td>CC</td>
<td>0.612</td>
<td>25.13±12.93</td>
<td>0.479</td>
<td>0.81±0.12</td>
<td>0.304</td>
<td>2.28 (4.28)</td>
<td>0.656</td>
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<tr>
<td></td>
<td></td>
<td>TCA</td>
<td>0.296</td>
<td>21.76±13.00</td>
<td>0.82±0.12</td>
<td>2.47 (3.65)</td>
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<tr>
<td></td>
<td></td>
<td>CGG</td>
<td>0.092</td>
<td>22.00±15.05</td>
<td>0.75±0.12</td>
<td>1.85 (3.12)</td>
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<tr>
<td>ABCB1</td>
<td>rs1128503</td>
<td>CC</td>
<td>0.316</td>
<td>26.25±14.97</td>
<td>0.467</td>
<td>0.78±0.12</td>
<td>0.143</td>
<td>2.26 (3.46)</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>0.469</td>
<td>22.36±11.17</td>
<td>0.81±0.13</td>
<td>2.28 (3.64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>0.224</td>
<td>23.09±14.12</td>
<td>0.84±0.12</td>
<td>2.76 (4.28)</td>
<td></td>
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<tr>
<td></td>
<td>rs2032582</td>
<td>GG</td>
<td>0.337</td>
<td>18.56±44.08</td>
<td>0.099</td>
<td>0.81±0.12</td>
<td>0.519</td>
<td>2.31 (3.35)</td>
<td>0.130</td>
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<tr>
<td></td>
<td></td>
<td>GA</td>
<td>0.031</td>
<td>42.68±19.13</td>
<td>0.78±0.18</td>
<td>1.94 (1.05)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GT</td>
<td>0.449</td>
<td>20.16±5.81</td>
<td>0.29±0.13</td>
<td>2.11 (4.28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA</td>
<td>0.061</td>
<td>29.29±42.44</td>
<td>0.83±0.10</td>
<td>2.92 (2.83)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>rs1049642</td>
<td>CC</td>
<td>0.327</td>
<td>20.00±55.96</td>
<td>0.874</td>
<td>0.80±0.12</td>
<td>0.749</td>
<td>2.27 (3.29)</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>0.510</td>
<td>23.13±54.81</td>
<td>0.80±0.13</td>
<td>2.24 (3.72)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>TT</td>
<td>0.163</td>
<td>21.71±46.63</td>
<td>0.83±0.12</td>
<td>2.74 (4.12)</td>
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</tr>
<tr>
<td>ABCB1</td>
<td>CGG</td>
<td>CC</td>
<td>0.694</td>
<td>24.46±13.36</td>
<td>0.780</td>
<td>0.80±0.12</td>
<td>0.247</td>
<td>2.27 (3.65)</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TTA/CGT</td>
<td>0.296</td>
<td>22.40±12.84</td>
<td>0.82±0.13</td>
<td>2.57 (4.28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 5% frequency</td>
<td>0.010</td>
<td>24.13±56.13</td>
<td>0.98±0.12</td>
<td>1.97 (3.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>PM</td>
<td>CC</td>
<td>0.073</td>
<td>23.76±13.77</td>
<td>0.901</td>
<td>0.83±0.13</td>
<td>0.229</td>
<td>3.33 (3.20)</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>0.271</td>
<td>25.87±14.80</td>
<td>0.76±0.45</td>
<td>2.25 (3.32)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>EM</td>
<td>0.625</td>
<td>23.54±12.20</td>
<td>0.82±0.62</td>
<td>2.27 (3.72)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>0.031</td>
<td>24.21±15.57</td>
<td>0.94±0.18</td>
<td>2.81 (1.17)</td>
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<td></td>
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</tr>
<tr>
<td>UGT2B7</td>
<td>rs7439366</td>
<td>CC</td>
<td>0.292</td>
<td>24.20±13.16</td>
<td>0.988</td>
<td>0.86±0.12</td>
<td>0.015</td>
<td>2.35 (3.40)</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>0.510</td>
<td>24.06±13.07</td>
<td>0.79±0.12</td>
<td>2.28 (3.85)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM</td>
<td>0.198</td>
<td>24.60±13.13</td>
<td>0.76±0.12</td>
<td>2.28 (3.43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPB1</td>
<td>rs1799971</td>
<td>AA</td>
<td>0.643</td>
<td>24.63±14.05</td>
<td>0.415</td>
<td>0.72±0.13</td>
<td>0.001</td>
<td>2.26 (4.28)</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
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<td>AG</td>
<td>0.286</td>
<td>21.25±10.41</td>
<td>0.87±0.092</td>
<td>2.47 (3.12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>0.071</td>
<td>27.16±14.13</td>
<td>0.86±0.10</td>
<td>2.47 (2.71)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ABCB1, ATP-binding cassette, sub-family B (MDR/TAP); COMT, catechol-O-methyltransferase; CYP2D6, cytochrome P450, family 2, subfamily D, polypeptide 6; EM, extensive metabolizer; IM, intermediate metabolizer; OPB1, opioïd receptor, mu 1; PM, poor metabolizer; SNP, single nucleotide polymorphism; UGT2B7, UDP glucuronosyltransferase 2 family, polypeptide B7; UM, ultrarapid metabolizer. Parametric data are expressed as mean ± s.d. and non-parametric data are expressed as median (range). Italicics values signifies statistical significance.
Figure 10. Mean dose (mg kg$^{-1}$) is expressed as mean ± s.d. (a) Mean dose (mg kg$^{-1}$) reported according to genotype at UGT2B7 rs7439366. N = 28, 49 and 19 for C/C, C/T and T/T, respectively. (b) Mean dose (mg kg$^{-1}$) reported according to genotype at OPRM1 rs1799971. N = 63, 28 and 7 for A/A, A/G and G/G, respectively. *P ≤ 0.05, **P ≤ 0.01.

Table 10. Multiple linear regression analysis to identify factors that predict mean codeine consumption reported by subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>$B$</th>
<th>SE</th>
<th>$\beta$</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT2B7 rs7439366</td>
<td>-0.054</td>
<td>0.016</td>
<td>-0.36</td>
<td>-0.086 to -0.021</td>
<td>0.002</td>
</tr>
<tr>
<td>OPRM1 rs1799971</td>
<td>0.068</td>
<td>0.018</td>
<td>0.34</td>
<td>0.032 to 0.10</td>
<td>0.000</td>
</tr>
<tr>
<td>OPRM1 rs1799971</td>
<td>0.112</td>
<td>0.037</td>
<td>0.466</td>
<td>0.048 to 0.20</td>
<td>0.002</td>
</tr>
</tbody>
</table>

4.3. Validating the Use of an LC-MS/MS Method in Hair

4.3.1. LC-MS/MS Parameters

Chromatographic separation and detection of FAEE present in hair using LC-MS/MS was achieved. The chromatograms derived from LC-MS/MS analysis of a neonatal hair sample are pictured in Figure 11. LC-MS/MS analysis generally produced chromatograms with visually discernable peaks. The FAEE source parameters were the following: curtain gas of 30 psi, ion spray voltage of 5000 V, source temperature of 600°C, gas 1 of 40 psi and gas 2 of 50 psi. The compound specific MS/MS parameters are described in detail in Table 11.
Figure 11. Chromatograms of the four FAEE analyzed in a neonatal hair sample using LC-MS/MS

Table 11. Compound specific MS/MS parameters for FAEE and d5-FAEE measured in neonatal hair

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>DP (V)</th>
<th>EP (V)</th>
<th>CE (V)</th>
<th>CXP (V)</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate-1</td>
<td>257.2</td>
<td>229.2</td>
<td>45</td>
<td>6</td>
<td>16</td>
<td>10</td>
<td>2.48</td>
</tr>
<tr>
<td>Myristate-2</td>
<td>257.2</td>
<td>103.0</td>
<td>45</td>
<td>6</td>
<td>22</td>
<td>13</td>
<td>N/A</td>
</tr>
<tr>
<td>Myristate-IS</td>
<td>262.3</td>
<td>230.2</td>
<td>90</td>
<td>6</td>
<td>17</td>
<td>22</td>
<td>2.46</td>
</tr>
<tr>
<td>Palmitate-1</td>
<td>285.3</td>
<td>257.4</td>
<td>45</td>
<td>5.5</td>
<td>16</td>
<td>10</td>
<td>3.46</td>
</tr>
<tr>
<td>Palmitate-2</td>
<td>285.3</td>
<td>71.0</td>
<td>45</td>
<td>5.5</td>
<td>24</td>
<td>10</td>
<td>N/A</td>
</tr>
<tr>
<td>Palmitate-IS</td>
<td>290.3</td>
<td>258.0</td>
<td>90</td>
<td>6</td>
<td>18</td>
<td>22</td>
<td>3.42</td>
</tr>
<tr>
<td>Oleate-1</td>
<td>311.3</td>
<td>265.3</td>
<td>55</td>
<td>5</td>
<td>16</td>
<td>23</td>
<td>3.89</td>
</tr>
<tr>
<td>Oleate-2</td>
<td>311.3</td>
<td>247.3</td>
<td>55</td>
<td>5</td>
<td>17</td>
<td>23</td>
<td>N/A</td>
</tr>
<tr>
<td>Oleate-IS</td>
<td>316.3</td>
<td>265.3</td>
<td>55</td>
<td>5.5</td>
<td>16</td>
<td>23</td>
<td>3.85</td>
</tr>
<tr>
<td>Stearate-1</td>
<td>313.2</td>
<td>285.4</td>
<td>80</td>
<td>5</td>
<td>17</td>
<td>10</td>
<td>4.18</td>
</tr>
<tr>
<td>Stearate-2</td>
<td>313.2</td>
<td>71</td>
<td>80</td>
<td>5</td>
<td>28</td>
<td>11</td>
<td>N/A</td>
</tr>
<tr>
<td>Stearate-IS</td>
<td>318.3</td>
<td>286.4</td>
<td>95</td>
<td>6</td>
<td>19</td>
<td>17</td>
<td>4.14</td>
</tr>
</tbody>
</table>

Analyses labeled with a “2” are qualifier transitions
Transitions derived from: Pichini et al., 2008; Kwak et al., 2010; Himes et al., 2014
DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision exit potential; N/A: not available
4.3.2. Validation Results, Extraction Efficiency and Matrix Effects

The LODs for myristate, palmitate, oleate and stearate were determined to be the following: 0.36, 0.31, 0.44 and 0.25 pg/mg, respectively (Table 12). The LOQs for the four FAEE, in the same respective order, were 1.2, 1.1, 1.5 and 0.8 pg/mg (Table 12). The linearity experiments revealed a %CV ranging from 3.9 to 10.1% for the slope comparisons and a %CV ranging from 0.11 to 0.67% for the regression line coefficient comparisons among the four FAEE (Table 12). These results suggest that the acceptance criterion for linearity was met. For myristate, palmitate and stearate, the upper limit of linearity was approximately 250 pg/mg (Table 12). The upper limit of linearity for oleate was approximately 500 pg/mg (Table 12).

Table 12. LOD, LOQ and linearity of FAEE analytes

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD (pg/mg)</th>
<th>LOQ (pg/mg)</th>
<th>Linear range (pg/mg)</th>
<th>Slope (%CV, n=3)</th>
<th>R² (%CV, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>0.36</td>
<td>1.2</td>
<td>1.2-250</td>
<td>3.9</td>
<td>0.67</td>
</tr>
<tr>
<td>Palmitate</td>
<td>0.31</td>
<td>1.1</td>
<td>1.1-250</td>
<td>10.1</td>
<td>0.38</td>
</tr>
<tr>
<td>Oleate</td>
<td>0.44</td>
<td>1.5</td>
<td>1.5-500</td>
<td>6.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Stearate</td>
<td>0.25</td>
<td>0.8</td>
<td>0.8-250</td>
<td>10.1</td>
<td>0.11</td>
</tr>
</tbody>
</table>

The specificity experiments, which examined a concentration of 75 pg/mg of each FAEE, revealed %CVs ranging between 0.24 and 0.80% for retention times and 2.6 to 17.9% for area ratios (Table 13). The extraction efficiencies of the four deuterated FAEE at 75 pg/mg each, ranged from 72 to 84% (Table 14). Matrix effects ranged between -25 and 54% (Table 14). The intra-day variability, which examined the calculated concentrations at 17.5 pg/mg and 75 pg/mg of each FAEE, was determined to be between 1.7 and 11.1% for the four FAEE (Table 15). This was similar to the inter-day variability %CVs, which ranged from 2.2 to 11.5% (Table 15). The %CV for accuracy and precision for 17.5 pg/mg and 75 pg/mg of each FAEE, ranged from 0.68 to 7.9% and from 0.59 to 4.0%, respectively. Therefore, the acceptance criteria for these two parameters were satisfied (Table 15). Carryover was not observed in mobile phase following the analysis of samples spiked at high concentrations (4000 and 12,000 pg/mg of FAEE mix).
Two hair EQC samples obtained from MEDICHEM Diagnostica (Steinenbronn, Germany) were tested and the percent recoveries for the four FAEE ranged between 85 and 102% (Table 15). Analysis of the external adult hair samples revealed acceptable agreement between the results obtained using the newly developed LC-MS/MS method and the reference values provided by MEDICHEM Diagnostica (Steinenbronn, Germany). The results from the LC-MS/MS – GC-MS comparability experiment also revealed that the two methods were highly correlated. All R-values were 0.987 or higher (Table 15).
Table 15. Intra and inter-day variability, accuracy, precision and percent recovery of EQC samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Intra-Day Variability (%CV, n=5)</th>
<th>Inter-Day Variability (%CV, n=20)</th>
<th>Accuracy (%CV; %Recovery, n=3)</th>
<th>Precision (%CV, n=3)</th>
<th>Percent Recovery of EQC Samples (%)</th>
<th>Methods Comparison (R-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>3.9</td>
<td>11.1</td>
<td>7.0</td>
<td>9.0</td>
<td>25; 100.8</td>
<td>0.98; 103.6</td>
</tr>
<tr>
<td>Palmitate</td>
<td>1.7</td>
<td>10.7</td>
<td>9.7</td>
<td>11.5</td>
<td>62; 100.3</td>
<td>4.6; 99.8</td>
</tr>
<tr>
<td>Oleate</td>
<td>2.8</td>
<td>8.1</td>
<td>5.5</td>
<td>7.4</td>
<td>71; 93.8</td>
<td>0.68; 98.0</td>
</tr>
<tr>
<td>Stearate</td>
<td>4.1</td>
<td>5.7</td>
<td>2.2</td>
<td>5.7</td>
<td>7.9; 96.5</td>
<td>1.8; 100.0</td>
</tr>
</tbody>
</table>

*Hair was spiked with 70 pg/mg and 300 pg/mg of FAEE mix

4.4. Establishing Baseline Levels of FAEE in Neonatal Hair

4.4.1. Baseline FAEE Results

Two hundred women participated in the baseline study. Among the 200 neonatal hair samples that were collected, 19 samples were not analyzed because they contained less than 20 mg of neonatal hair, and 4 patients were excluded because they did not meet the inclusion criteria. Of the remaining 177 neonatal hair samples that underwent LC-MS/MS analysis, one sample could not be successfully analyzed. Inspection of the Levey-Jennings QC chart revealed that all QC samples (n=15) except one were within two standard deviations of the mean. The results of the FAEE analysis in neonatal hair are summarized in Table 16. Given that the FAEE concentrations for a proportion of samples were below the LOQ for all four analytes, quantitative results are not available for two samples analyzed. The mean concentrations of the individual FAEE were approximately equal. The median concentrations of palmitate and oleate were slightly higher than the levels of myristate and stearate. The combined concentrations of FAEE and their respective percentile plots are presented in Table 16 and Figure 12, respectively.
Table 16. Summary of baseline FAEE results measured in neonatal hair samples

<table>
<thead>
<tr>
<th>Analyte(s)</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate (pg/mg), n=114</td>
<td>2.9 ± 5.8</td>
<td>1.7</td>
<td>1.2</td>
<td>61</td>
</tr>
<tr>
<td>Palmitate (pg/mg), n=173</td>
<td>2.7 ± 1.4</td>
<td>2.3</td>
<td>1.1</td>
<td>11</td>
</tr>
<tr>
<td>Oleate (pg/mg), n=69</td>
<td>2.6 ± 1.7</td>
<td>2.1</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td>Stearate (pg/mg), n=173</td>
<td>2.1 ± 2.0</td>
<td>1.6</td>
<td>0.8</td>
<td>23</td>
</tr>
<tr>
<td>Palmitate (pg/mg) + Stearate (pg/mg), n=172</td>
<td>4.7 ± 2.8</td>
<td>4.0</td>
<td>2.1</td>
<td>25</td>
</tr>
<tr>
<td>Myristate (pg/mg) + Palmitate (pg/mg) + Stearate (pg/mg), n=114</td>
<td>8.1 ± 6.8</td>
<td>6.4</td>
<td>3.3</td>
<td>66</td>
</tr>
<tr>
<td>Total of all four (pg/mg), n=50</td>
<td>12.7 ± 9.6</td>
<td>9.5</td>
<td>6.1</td>
<td>68</td>
</tr>
</tbody>
</table>

Figure 12. Percentile plots for combinations of FAEE in the hair of neonates exposed to one drink or less per week during the 3rd trimester
4.4.2. Clinical Characteristics and Associations with FAEE Concentrations

The clinical characteristics (Appendix F), of the 177 subjects included in the baseline study along with the p-values derived from the univariate analyses are described in Table 17. The adjusted Bonferroni critical value was 0.0036. The data exhibited a non-normal distribution. Approximately 21% of the participants exhibited a low-level social drinking behavior in the third trimester of pregnancy, consuming an average of 1.66 ± 2.21 drinks in total. Drinking in the third trimester was not associated with levels of FAEE in the hair. The measured FAEE concentrations also did not differ between hair samples collected from neonates who had a bath prior to sample collection and those who did not. None of the individual variables were associated with the FAEE levels in neonatal hair, although some approached significance (p-value of less than 0.050) (Table 17). The association between maternal medical condition during pregnancy and the concentration of the four FAEE combined approached significance (U=359, z=2.312, p=0.021). Neonates born to mothers with a medical condition had a higher median level of FAEE in their hair (12.2 pg/mg) compared to those born to healthy mothers (9.1 pg/mg). The relationship between neonatal complications and the level of palmitate (U=1119, z=1.977, p=0.048), stearate (U=1129.50, z=2.046, p=0.041), and the combined concentration of palmitate and stearate (U=1110.50, z=1.966, p=0.049) approached significance. It was found that neonates born with complications had higher median levels of palmitate (3.17 vs. 2.24 pg/mg), stearate (2.04 vs. 1.62 pg/mg) and combined concentrations of palmitate and stearate (4.91 vs. 3.96 pg/mg) than neonates without complications. The association between the concentration of palmitate in hair and the number of neonatal antibiotic treatment days was also close to reaching significance, whereby an increasing number of antibiotic treatment days was linked to higher levels of palmitate in neonatal hair (N=173, r=0.151, p=0.048).

Lack of normality in the data precluded the completion of multivariate analyses. However, one of the variables (maternal medical condition) that approached significance was further explored. To investigate the relationship between maternal medical condition and levels of the four FAEE combined, statistical analyses were conducted to determine whether the three most common conditions among subjects (diabetes, hypothyroidism and asthma)
were associated with the total FAEE concentration. Diabetes came closest to being associated with said dependent variable (U=143, z=1.824, p=0.070), while the other two independent variables, hypothyroidism (U=129, z=0.534, p=0.615) and asthma (U=87, z=-0.179, p=0.877) did not come close. The associations between neonatal complications and various FAEE concentrations were not further examined, because there was too much heterogeneity in the types of complications. An in-depth examination of the link between neonatal antibiotics and palmitate levels could not be performed, because almost all neonates were taking the same antibiotics.

Table 17. Clinical characteristics of baseline study population and results of univariate analyses

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Mean</th>
<th>Myristate (pg/mg)</th>
<th>Palmitate (pg/mg)</th>
<th>Oleate (pg/mg)</th>
<th>Stearate (pg/mg)</th>
<th>Palmitate + Stearate (pg/mg)</th>
<th>Myristate + Palmitate + Oleate + Stearate (pg/mg)</th>
<th>Total of all four (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.83 ± 4.88</td>
<td>0.469</td>
<td>0.758</td>
<td>0.345</td>
<td>0.154</td>
<td>0.463</td>
<td>0.654</td>
<td>0.804</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.42 ± 1.54</td>
<td>0.755</td>
<td>0.280</td>
<td>0.097</td>
<td>0.082</td>
<td>0.185</td>
<td>0.356</td>
<td>0.758</td>
</tr>
<tr>
<td>Drinking 3rd trimester</td>
<td>Yes</td>
<td>0.089</td>
<td>0.137</td>
<td>0.177</td>
<td>0.682</td>
<td>0.272</td>
<td>0.096</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.140 (79.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of drinks 3rd trimester</td>
<td>1.66 ± 2.21</td>
<td>0.071</td>
<td>0.123</td>
<td>0.209</td>
<td>0.584</td>
<td>0.238</td>
<td>0.076</td>
<td>0.941</td>
</tr>
<tr>
<td>Maternal Infection GBS positive</td>
<td></td>
<td>0.516</td>
<td>0.590</td>
<td>0.995</td>
<td>0.907</td>
<td>0.960</td>
<td>0.444</td>
<td>0.746</td>
</tr>
<tr>
<td></td>
<td>GBS-ve/unknown</td>
<td>41 (23.2)</td>
<td>136 (76.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal antibiotics</td>
<td>Yes</td>
<td>0.614</td>
<td>0.847</td>
<td>0.880</td>
<td>0.875</td>
<td>0.778</td>
<td>0.284</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.847 (52.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal antibiotic treatment days</td>
<td>1.13 ± 0.34</td>
<td>0.577</td>
<td>0.710</td>
<td>0.993</td>
<td>0.772</td>
<td>0.669</td>
<td>0.177</td>
<td>0.544</td>
</tr>
<tr>
<td>Medical Condition</td>
<td>Yes</td>
<td>0.260</td>
<td>0.681</td>
<td>0.491</td>
<td>0.344</td>
<td>0.625</td>
<td>0.453</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.215 (78.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td>Yes</td>
<td>0.351</td>
<td>0.653</td>
<td>0.760</td>
<td>0.946</td>
<td>0.968</td>
<td>0.935</td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.169 (83.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy/labor complications</td>
<td>Yes</td>
<td>0.784</td>
<td>0.904</td>
<td>0.563</td>
<td>0.913</td>
<td>0.794</td>
<td>0.855</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.167 (83.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal complications</td>
<td>Yes</td>
<td>0.073</td>
<td>0.048</td>
<td>0.243</td>
<td>0.041</td>
<td>0.049</td>
<td>0.093</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.067 (94.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal antibiotics</td>
<td>Yes</td>
<td>0.085</td>
<td>0.050</td>
<td>0.776</td>
<td>0.556</td>
<td>0.156</td>
<td>0.351</td>
<td>0.376</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.067 (96.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal antibiotic treatment days</td>
<td>Yes</td>
<td>2.0 ± 1.10</td>
<td>0.081</td>
<td>0.048</td>
<td>0.719</td>
<td>0.535</td>
<td>0.150</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>132 (74.6)</td>
<td>45 (25.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GBS: group B streptococcus
-ve: negative
Chapter 5
DISCUSSION

5.1. Pharmacogenetics of Opioid Therapy in the Management of Postpartum Pain: A Systematic Review

5.1.1. Quality of Studies

Most of the cohort studies lost stars for not having a non-exposed cohort. This flaw prevented them from being assessed with respect to comparability of exposed and non-exposed cohorts (Xu et al., 2015; VanderVaart et al., 2011a; Kelly et al., 2013; Baber et al., 2015; Sia et al., 2008; Tan et al., 2009; Sia et al., 2010; Tsai et al., 2010; Wong et al., 2010; Boswell et al., 2013; Stauble et al., 2014; Zhang et al., 2013). One cohort study contained a non-exposed cohort, but failed to match and/or adjust for confounders between exposed and non-exposed cohorts (Ciszkowski et al., 2010). Many cohort studies also lost stars for relying on self-reported data or for lacking a description for the assessment of outcome (Ciszkowski et al., 2010; Xu et al., 2015; VanderVaart et al., 2011a; Kelly et al., 2013; Baber et al., 2015; Sia et al., 2008; Tan et al., 2009; Sia et al., 2010; Tsai et al., 2010; Wong et al., 2010; Boswell et al., 2013; Stauble et al., 2014; De Capraris et al., 2011; Zhang et al., 2013) and the ascertainment of exposure (Xu et al., 2015; VanderVaart et al., 2011a; Baber et al., 2015; Sia et al., 2008; Tan et al., 2009; Sia et al., 2010; Wong et al., 2010; Zhang et al., 2013). Finally, many cohort studies failed to demonstrate that the outcome of interest was not present at the start of the study (Ciszkowski et al., 2010; VanderVaart et al., 2011a; Kelly et al., 2013; Baber et al., 2015; Sia et al., 2010; Wong et al., 2010; Boswell et al., 2013; Stauble et al., 2014; De Capraris et al., 2011; Zhang et al., 2013). Specifically, studies should have ensured the exclusion of women who had taken an analgesic prior to the start of the study, since this can confound the results.

All case–control studies lost stars for establishing cases via self-report, ascertaining exposure through an interview that was not blinded to case/control status and failing to report no history of outcome for controls. These studies may have thereby lacked appropriate selection of cases and controls (Lam et al., 2013; Madadi et al., 2009; Sistonen et al., 2012).
Two of the case–control studies also neglected to match cases and controls and/or adjust for confounders (Lam et al., 2013; Madadi et al., 2009). Another issue among the case–control studies pertains to the representativeness of the cases (Lam et al., 2013; Madadi et al., 2009; Sistonen et al., 2012). There is potential for selection bias given that the cases likely consist of highly motivated women – who chose to call a telephone counseling service and subsequently agreed to participate in research.

Overall, most of the studies were of low quality (13/17), with the exception of four studies that were of moderate quality. The majority of the studies lost stars for the use of self-reported information to ascertain exposure or outcome, which is subject to bias. Given the nature of pain research, studies generally need to rely on self-reporting to assess analgesia and adverse events. Many studies also lost stars for not having an unexposed cohort. This meant that they were unable to earn stars on comparability between exposed and non-exposed cohorts. In order to assess the role of specific genetic variants on pain relief and side effects following opioid intake, however, all subjects necessarily have to be exposed to the drug and genotyped. Therefore, there may not always be an appropriate unexposed cohort in pharmacogenetic studies. It is also worth noting that much of the research is done in a ‘real-life’ clinical setting, and although this type of research may contain bias and methodological issues, it can also provide investigators with a unique opportunity for exploratory learning. The findings that arise from this early exploratory work can go on to inform future clinical studies that are more rigid in their methodology.

The few studies that examine the role of opioid pharmacogenetics in postpartum pain are underpowered or of low-to-moderate quality. Hence, this systematic review of the literature might be useful for providing strength to the individual studies via mutual corroboration. Similar findings among several studies help to build a narrative on the role of genetic polymorphisms, and can assist in focusing future studies on genetic markers that likely play a role in opioid analgesia. At the same time, a comprehensive analysis of the deficiencies among the studies can help to improve future study designs.
5.1.2. CYP2D6

The significant findings associated with CYP2D6 in this review are consistent with many published works related to the gene. It has been well established in the literature that the CYP2D6 polymorphism is involved in codeine analgesia and adverse events following opioid intake. The CYP2D6 PM phenotype is associated with decreased morphine production compared with EM and UM, leading to decreased pain relief (Lötsch et al., 2009a). On the other hand, CYP2D6 UM status can predispose a patient to adverse reactions, even at low codeine doses, such as dizziness, sedation, nausea and respiratory depression (Crews et al., 2012; Gasche et al., 2004). To address the variability to codeine response, Crews et al. developed dosing guidelines according to CYP2D6 phenotype (2012). Similar to codeine, hydrocodone/hydromorphone (Otton et al., 1993; Barakat et al., 2012) and oxycodone/oxymorphone ratios (Andreassen et al., 2012; Zwisler et al., 2010a) in the body vary according to CYP2D6 status. However, the phenotypic differences that arise from the CYP2D6 phenotype with respect to analgesia and adverse effects have not been clearly defined following hydrocodone (Otton et al., 1993; Kaplan et al., 1997; Susce et al., 2006) and oxycodone intake (Andreassen et al., 2012; Zwisler et al., 2009).

Among the four studies that examined the role of CYP2D6 in analgesia among post-cesarean patients prescribed codeine or hydrocodone, none found statistically significant associations. Three studies described a trend (VanderVaart et al., 2011a; Baber et al., 2015; Stauble et al., 2014). One codeine study found that its PMs consumed more codeine than the other phenotypes (Baber et al., 2015). Another codeine study indicated that its UMs reported immediate analgesia, while its PMs consumed the most codeine and did not experience analgesia (VanderVaart et al., 2011a). Similarly, the hydrocodone study found that its PMs consumed the most codeine (Stauble et al., 2014). Given the small number of extreme phenotypes in all of the above studies, the role of CYP2D6 in postpartum analgesia is unclear. However, most studies reported that its PMs consumed more opioid, which is consistent with the notion that PMs experience inadequate analgesia due to decreased opioid metabolism.

Seven studies examined the role of CYP2D6 in side effects experienced by mother and/or infant. Two studies reported a lack of association between CYP2D6 and adverse events
following codeine (Kelly et al., 2013) or hydrocodone intake (Stauble et al., 2014). Two studies had reports of CNS depression in infants among mothers that were UMs after taking codeine (Ciszkowski et al., 2010) or oxycodone (Lam et al., 2013). One study reported dizziness and constipation among its UMs who took codeine (VanderVaart et al., 2011a). The only two studies that had statistically significant findings demonstrated an association between CYP2D6 phenotypic status and CNS depression in infant (Madadi et al., 2009; Sistonen et al., 2012) or mother (Sistonen et al., 2012) following maternal intake of codeine. These studies suggest that CYP2D6 UM status may be a significant contributor to adverse events that arise in mother and infant, or infant alone, following opioid exposure. In particular, it may contribute to the manifestation of CNS depression due to increased morphine production.

5.1.3. OPRM1 A118G

The significant findings related to OPRM1 A118G in this review are consistent with the proposed biological significance of the variant. In vitro studies suggest that the 118G allele is associated with reduced receptor expression leading to decreased potency and efficacy following exposure to opioids (Mahmoud et al., 2011; Zhang et al., 2005). Recently published work is also consistent with the outcomes associated with OPRM1 A118G. A recent meta-analysis on the role of OPRM1 A118G in the postoperative period demonstrated that 118G carriers had a lower incidence of nausea and vomiting, higher pain scores and consumed more opioid than 118A homozygotes within the first 24 h postoperative period (Ren et al., 2015). Another meta-analysis exploring the relationship between OPRM1 A118G and postoperative opioid requirement similarly found that 118G carriers required a higher mean opioid dose than 118A homozygotes (Hwang et al., 2014).

Eight studies examined the role of OPRM1 A118G in analgesia. Both sufentanil studies, and one of the hydrocodone studies did not find an association between the gene variant and reported pain scores (Xu et al., 2015; De Capraris et al., 2011), dose intake (Xu et al., 2015; Wong et al., 2010) or satisfaction scores (Xu et al., 2015; Wong et al., 2010). On the other hand, four studies (one codeine, two morphine and one fentanyl) demonstrated a significant association between OPRM1 A118G and analgesia. In general, it was found that the 118G allele was associated with greater drug intake (Baber et al., 2015; Sia et al., 2008; Tan et al.,
2009; Zhang et al., 2013) and higher reported pain scores (Sia et al., 2008; Tan et al., 2009; Zhang et al., 2013). Another study administering hydrocodone for pain found that pain levels were inversely correlated with total hydrocodone dose and serum hydromorphone levels for individuals with the AA genotype, but not for those with the AG/GG genotype (Boswell et al., 2013). There are conflicting findings with respect to the relevance of the OPRM1 A118G variant in modulating analgesia following opioid intake. However, among the findings that demonstrate an association, the effect of the variant appears to be similar among studies.

Ten studies examined the role of the OPRM1 A118G variant in adverse events in the mother. Half of the studies (two codeine, one oxycodone, one fentanyl and one sufentanil) did not find a significant relationship between this polymorphism and adverse events (Lam et al., 2013; Xu et al., 2015; Sistonen et al., 2012; Kelly et al., 2013; Zhang et al., 2013). Three studies in which women received morphine found that the 118G allele was associated with reduced side effects, such as nausea (Sia et al., 2008; Tan et al., 2009), vomiting (Tan et al., 2009) and pruritus (Tsai et al., 2010). In a study in which patients received hydrocodone, 118G carriers similarly had a reduced risk of pruritus (Wong et al., 2010). However, another hydrocodone study showed the opposite association, whereby individuals with 118G allele had a higher risk for side effects, such as constipation, vomiting and respiratory depression (Boswell et al., 2013). Similar to the analgesia findings for OPRM1 A118G, the studies are split with respect to the involvement of the variant in adverse events. However, among the studies that found a significant relationship, the majority agreed in terms of the influence of the variant. These studies suggest that OPRM1 118G is associated with less effective opioid analgesia and reduced side effects compared with wild-type 118A.

5.1.4. UGT2B7 C802T

The biological significance of the UGT2B7 C802T variant with respect to opioid response is controversial. Some of the literature suggests that the T allele is associated with higher M6G/morphine plasma ratios (Sawyer et al., 2003), which leads to improved analgesia due to the increased potency of M6G (Portenoy et al., 1992). Other published findings have revealed no relationship between genotype and M6G/morphine ratios (Holthe et al., 2003), and a lack of improved analgesia associated with M6G (Lötsch et al., 1997). If the former
finding is accurate, it would provide a biological explanation for the significant associations discussed in this review that are relevant to the \textit{UGT2B7} C802T variant. On the other hand, there are contrary findings in the literature. For instance, a Japanese study found that the frequency of nausea in 802T carriers is lower compared with non-carriers among cancer patients (Fujita et al., 2010).

In this review, one study examined the role of \textit{UGT2B7} C802T in codeine analgesia (Baber et al., 2015). It found that TT individuals consumed less codeine on average than CC individuals. It also found that together with \textit{OPRM1} A118G, this variant predicts mean dose consumption in the cohort overall.

Three codeine studies examined the role of \textit{UGT2B7} C802T in adverse events in mother and infant, or infant alone. Two studies did not find a significant association between the variant and CNS depression or sedation in mother or infant (Sistonen et al., 2012; Kelly et al., 2013). One codeine study found that a combination of CYP2D6 UM status and the \textit{UGT2B7} 802TT genotype in the mother puts the infant at an increased risk for CNS depression (Madadi et al., 2009). The significant findings on \textit{UGT2B7} C802T in this review are consistent with the notion that 802T is associated with the increased formation of M6G, which leads to a decreased opioid requirement among postpartum patients (Baber et al., 2015) and an increased risk for CNS depression among mothers and their breastfeeding infants (Madadi et al., 2009).

5.1.5. \textit{ABCB1} polymorphisms (C1236T, G2677AT, C3435T)

\textit{ABCB1} 3435T has been associated with the reduced expression and activity of P-gp (Somogyi et al., 2007; Hoffmeyer et al., 2000). Since P-gp functions as a drug efflux transporter at the blood–brain barrier, a reduction in its activity would be expected to lead to increased drug concentrations in the brain. Consistent with this biological explanation, studies have found that 3435T homozygotes report greater pain relief following opioid consumption (Campa et al., 2008). Furthermore, 3435T homozygotes or carriers have been found to require lower doses of opioids (Lötsch et al., 2009b; Gong et al., 2013). \textit{ABCB1} G2677AT is in strong linkage disequilibrium with C3435T and has been similarly linked to varying P-gp
activity by impairing clearance of drugs out of target cells (Sakurai et al., 2007). However, its effect on P-gp expression levels is unknown (Lam et al., 2013). In the literature, the role of ABCB1 G2677AT in adverse events following opioid intake is also unclear. One study found no association between the variant and drowsiness. However, it found that the variant was associated with more headaches, but less severe nausea and vomiting compared with wild type (Zwisler et al., 2010b). A Japanese study found that the TT/TT diplotype (combining ABCB1 C3435T and G2677AT) was associated with a lower frequency of side effects among cancer patients (Fujita et al., 2010). Despite the disparate findings in the literature, the significant findings related to ABCB1 G2677AT in this review are consistent with the proposed biological consequence of this variant (Sakurai et al., 2007).

Two studies investigated the role of three ABCB1 polymorphisms (ABCB1 C1236T, G2677AT, C3435T) in analgesia. A codeine study did not find any significant association (Baber et al., 2015), however, a morphine study found that 3435T homozygotes had the longest mean survival time of wound pain compared with CT and TT individuals (Sia et al., 2010). It would appear that this finding is in tension with the proposed role of 3435T. It is expected that 3435T homozygotes would experience less pain due to increased concentrations of morphine in the brain. Sia et al. (2010) reconciled its disparate finding that the TT genotype is associated with longer wound pain by suggesting that greater exposure to opioids may lead to increased pain sensitivity (Angst et al., 2003). However, they admit that this has yet to be linked to reduced ABCB1 function.

Four studies examined the influence of ABCB1 polymorphisms in adverse events. Two studies (one codeine and one morphine) did not demonstrate any significant associations (Kelly et al., 2013; Sia et al., 2010). However, the oxycodone study showed that mothers who carried at least one copy of the ABCB1 2677T allele were at an increased risk for experiencing sedation (Lam et al., 2013). Similarly, a codeine study found that CYP2D6 UM status in combination with the ABCB1 2677TT genotype is associated with CNS depression in mother and infant (Sistonen et al., 2012). The ABCB1 2677T variant may be a contributing factor associated with sedation in the mother or CNS depression in both mother and infant due to the increased accumulation of opioid in the brain.
5.1.6. Limitations

Understanding the limitations of pharmacogenetic studies can assist in understanding the significance of the findings and reconciling contradictory results among studies. One of the major confounders associated with comparing pharmacogenetic studies is that the nature of the pain or the indication for the opioid may be different. For instance, it may not be appropriate to compare the pain and opioid consumption between cancer patients and postpartum patients. One of the highlights of this review is that it contains a homogenous patient population with respect to opioid indication. Although, it should be noted that three studies included in this review contain a proportion of breastfeeding patients that were treated for other indications, but not excluding postpartum pain (Lam et al., 2013; Madadi et al., 2009; Sistonen et al., 2012). Therefore, the results of these studies should be interpreted with caution with respect to their applicability to the postpartum population. It should be noted that there are also differences in the type of C-section that is conducted (i.e., different kinds of incisions) and the nature of analgesia used during the procedure, which can contribute to differences among studies. Given that opioids differ with respect to their action in the body, the type of opioid that is prescribed to patients postpartum can also lead to disparate findings among studies. For example, different opioids that act on the MOR may have subtle pharmacological differences, leading to differences in their potency, effectiveness and tolerability among patients (Pasternak, 2012).

Methodological differences also limit effective comparison of the studies. Each type of study design is subject to unique biases that may play a role in the nature of the results. Inadequate sample size may have caused some studies to be underpowered to show a statistically significant association. For instance, some of the published work exploring the role of CYP2D6 contained very few extreme phenotypes (VanderVaart et al., 2011a; Kelly et al., 2013; Baber et al., 2015; Stauble et al., 2014), which suggests that they were likely underpowered to show significance. Studies also differ with respect to how long their patients are followed-up after surgery (i.e., 24 h vs 48 h postoperatively). There are also differences in the methods used to assess pain and the frequency at which it is assessed. The route of drug administration, the doses that are prescribed and how dose intake is calculated
(i.e., mg vs mg/kg; mean vs cumulative) also differ. Another issue to consider is that dose intake was patient-controlled in many studies; therefore, this may limit effective interpretation of the pharmacogenetic findings. For instance, some women may have purposely limited their opioid intake, fearing that it may cause adverse effects in themselves and their infants.

Finally, it is important to keep in mind the significant role of non-genetic determinants that account for differences among patients within studies and between studies. As discussed earlier, age, ethnicity, comorbidities, co-medication and psychological differences represent only a fraction of factors that contribute to the outcomes related to postpartum pain management. It should be noted that in the context of pain therapy, there is a complex interplay of genetic and non-genetic factors involved in opioid response.

5.2. Pharmacogenetics of Codeine Pain Relief in the Postpartum Period

5.2.1. Clinical and Pharmacogenetic Findings and Their Relation to the Literature

This study offers insight into the potential role of maternal age in modulating analgesic response, and the roles of OPRM1 A118G and UGT2B7 C802T polymorphisms in altering the amount of postpartum codeine required. This study also provides evidence of a potential link between ethnic differences in opioid consumption and genetic polymorphisms in the codeine pathway, which may partially account for said differences. Together, these findings point to the need for individualized therapy in managing postpartum pain with codeine.

In this nested cohort, increasing maternal age was predictive of higher pain scores. Previous studies have shown that pain intensity following C-sections decreases with increasing maternal age and that older patients experience greater pain relief following the same dose of medication (VanderVaart et al., 2011a; VanderVaart et al., 2011b; Bellville et al., 1971). Conversely, in this cohort, increasing maternal age was predictive of higher pain scores following codeine intake. Although one might expect an opposite effect of age on pain sensitivity, baseline pain measurements prior to codeine intake were not collected and codeine consumption was not controlled. Even though maternal age was not associated with
codeine requirements in this cohort, older women may have needed less codeine (due to lower initial pain following C-section) and, therefore, may have experienced more pain relative to younger women who took more codeine (due to greater initial pain following C-section).

In our study, polymorphism at the MOR was shown to influence mean codeine intake in participants. This finding is in line with the hypothesis that individuals carrying a copy of the variant require a larger dose of codeine to achieve analgesia, due to decreased receptor expression. Sia et al. (2008) and Tan et al. (2009) similarly found that subjects homozygous for the *OPRM1* 118G variant needed more morphine to manage postpartum pain following C-section. Conversely, Janicki et al. (2006) and Coulbault et al. (2006) found that the 118G variant did not affect morphine intake among subjects with postoperative pain. The discrepancies observed between studies may be attributed to distinct genetic and clinical features characterizing the various study populations.

To further elucidate the role of *OPRM1* A118G polymorphism, Mahmoud et al. subjected humanized sensory neurons to morphine (2011). In neurons homozygous for the G allele, morphine was roughly five times less potent and exhibited 26% less efficacy compared to those homozygous for the A allele. Molecular modeling completed by Zhang et al. suggests that this variant causes reduced receptor expression via its deleterious effects on mRNA and protein production (2005). Together, these findings support the observation that individuals carrying the 118G variant might require a greater mean dose of codeine than those without the variant.

Polymorphism at *UGT2B7* rs7439366 (C802T) was also found to influence mean codeine intake. The role of *UGT2B7* C802T variant in altering morphine glucuronidation is not fully understood. Sawyer et al. have shown that among patients receiving intravenous morphine postoperatively, individuals with the TT genotype had the highest M6G/morphine plasma ratio followed by CT and CC individuals (2003). Furthermore, morphine levels were lowest among TT individuals, while M6G levels were lowest among CC individuals. Conversely, a study examining cancer patients on chronic morphine therapy revealed that the glucuronide to morphine ratios did not differ among genotypic groups of the *UGT2B7* C802T variant.
The role of M6G in analgesia has also been controversial. Despite being less efficiently diffused across the blood–brain barrier compared to morphine, M6G is more potent, which is likely responsible for its clinical effects. Portenoy et al. examined the relationship between M6G to morphine ratio and the extent of pain relief following an infusion of morphine among chronic pain patients (1992). The study revealed that individuals carrying the highest ratio of M6G to morphine experienced the greatest extent of analgesia. In contrast, a study involving the intravenous administration of M6G with and without morphine revealed that M6G was not associated with analgesia on its own or in combination with morphine (Lötsch et al., 1997).

If it is assumed that the presence of the 802T variant produces a higher ratio of M6G to morphine relative to wild-type homozygous and that this higher ratio is associated with increased pain relief, we can infer why homozygous T individuals required less codeine than homozygous C individuals.

5.2.2. Genetically-Mediated Ethnic Differences in Codeine Requirement

Several studies that have explored the relationship between ethnicity and postoperative opioid consumption have reported results antithetical to ours, wherein individuals of Asian descent were found to consume less analgesic than Caucasians (Carnie and Perks, 1984; Houghton et al., 1992; Konstantatos et al., 2012). Differences in opioid consumption seen between these two ethnicities may be, in part, related to differences in allele frequencies for the OPRM1 rs1799971 and UGT2B7 rs7439366 polymorphisms. There is a difference in the allelic frequency of the OPRM1 118G variant, which is close to 50% among Asians and 10–15% among Caucasians (Sadhasivam and Chidambaran, 2012). Interestingly, in this nested cohort, the frequency of the G allele was ~ 45% among Asians (n = 15) and 12% among Caucasians (n = 38). For the UGT2B7 C802T variant, the prevalence of C allele among Asians was 73% and only 46% among Caucasians (Lampe et al., 2000). Similarly, the allelic frequency of C allele in our Asian and Caucasian populations was roughly 68% and 46%, respectively. Multivariate analysis revealed that both polymorphisms are predictive of mean
codeine intake among Caucasians; however, this relationship was not found among Asians. This may be due to the small sample of Asians in the study. It can be inferred that the differences in mean dose intake seen between Asians and Caucasians may be attributable, in part, to differences in allelic frequencies for the \textit{OPRM1} A118G and \textit{UGT2B7} C802T variants.

The finding that \textit{OPRM1} and \textit{UGT2B7} predict codeine intake in the cohort overall, along with the finding of a difference in mean codeine consumption between various ethnicities, prompted an investigation into the role of these polymorphisms in Asians and Caucasians. Given that there are significant differences in the reported allelic frequencies for these two polymorphisms, between Asians and Caucasians, logically we felt it would be worth exploring whether these polymorphisms are predictive of codeine intake in these two populations. The small number of patients in the ethnic categories is somewhat limiting. However, it was shown that the allelic differences reported in the literature existed between our Caucasian and Asian patients. Further, the initial finding that the two polymorphisms play a role in codeine intake in the cohort overall, serves to corroborate the ethnic finding that the polymorphisms predict codeine consumption among Caucasians. Similarly, the ethnic finding helps to corroborate the initial finding. Hence, these two pieces of evidence are mutually supportive and especially useful when understood together. Of course, though these findings are statistically significant, the extent to which they may affect clinical practice must still be considered.

5.2.3. Clinical Application, Other Determinants of Analgesia, and Limitations

This study explores the potential impact of \textit{OPRM1} and \textit{UGT2B7} polymorphisms in modulating codeine responsiveness among women being treated for postpartum pain following C-section. The literature is somewhat inconsistent with respect to the role of these two polymorphisms in opioid analgesia. This may be expected given that there are multiple determinants of codeine responsiveness, and the clinical features characterizing a cohort varies between study populations. Despite the complexities associated with interpreting pharmacogenetic findings, this study offers important and mutually corroborative pieces of evidence that \textit{OPRM1} and \textit{UGT2B7} play a role in codeine analgesia.
In the cohort overall and among Caucasians, the genes account for 18.4% and 28% of the inter-individual variation in codeine consumption, respectively. Differences in mg per kg of codeine consumed between the genotypic groups of OPRM1 and UGT2B7 are relatively small. Although these polymorphisms do not play a particularly large role in determining codeine requirement, their impact may be significant depending on the clinical context. Combined with other factors, the genotype of individuals at these two polymorphisms might be helpful for optimizing therapy for a specific patient. For instance, a woman with CYP2D6 IM phenotype, and who carries the UGT2B7 and OPRM1 genotypes associated with a greater codeine requirement, may be prescribed a larger dose of codeine than a woman with CYP2D6 EM phenotype, and who carries the UGT2B7 and OPRM1 genotypes associated with a smaller codeine requirement. Given that these two polymorphisms do not account for a particularly substantial portion of the inter-individual variability in codeine intake, other determinants of codeine responsiveness should be considered.

With respect to genetic determinants of opioid analgesia, the CYP2D6 phenotype is known to play an important role (Crews et al., 2012). In our cohort, PMs consumed more codeine cumulatively than IMs, EMs and UMs, although this was not quite statistically significant. It is also possible that COMT and ABCB1 polymorphisms have an effect on drug responsiveness, but this study was not sufficiently powered to detect differences between the genotypic groups.

Among non-genetic determinants, age is an important factor contributing to interindividual variability in drug response, and was found to be predictive of mean pain score reported by patients. Physiological changes that occur over time can have an impact on the pharmacokinetics and pharmacodynamics of drugs (Madadi and Koren, 2012). Adherence may have also contributed to differences between patients (Madadi and Koren, 2012). Some patients may have been concerned about adverse effects of codeine intake to themselves, or to their babies (through breastfeeding). These concerns may have prompted some patients to limit their consumption of codeine. Other factors that may impact codeine responsiveness include disease states, previous C-sections or surgeries, variability in pain sensitivity, emotional states and attitudes toward pain (Madadi and Koren, 2012; Kim et al., 2013; Yang
et al., 2000).

Our study has limitations that should be acknowledged. Baseline pain measurements were not recorded and codeine consumption was variable between participants. At the time of receiving their codeine prescription, subjects were provided safety guidelines, which recommended that they try to limit their codeine intake to 4 days in order to reduce the risk of serious CNS depression in their babies while breastfeeding. It is likely that some women limited their codeine intake as a result of these guidelines, and hence their reported codeine consumption and pain perception may not be true reflections of otherwise uninfluenced codeine intake. Irrespective of these limitations, this study provides insight into genetic markers that may assist in individualizing codeine therapy during the postpartum period following C-section.

5.3. Validating the Use of an LC-MS/MS Method in Hair

5.3.1. Improved Analytical Method

The few studies that have been published on FAEE analysis in hair involve the use of GC-MS (Auwärter et al., 2001; Pragst et al., 2001; Kulaga et al., 2009). LC-MS/MS analysis significantly increased analytical sensitivity, which facilitated the quantification of FAEE in neonatal hair samples. The LOQs of the GC-MS method range from 40 to 120 pg/mg (Pragst et al., 2001), whereas the LOQs for the LC-MS/MS method range from 0.8 to 1.5 pg/mg (Table 12). The latter method is significantly more sensitive than the former method. Interestingly, the relative sensitivities of the four analytes are similar to the GC-MS method (Pragst et al., 2001), with stearate exhibiting the highest sensitivity (lowest LOQ) and oleate exhibiting the lowest sensitivity (highest LOQ). Given that the median concentrations of individual FAEE in neonatal hair samples ranged from 1.6 to 2.3 pg/mg, these low levels would not be quantifiable using the former GC-MS method (Pragst et al., 2001). Moreover, quantification of the neonatal hair samples allows for a statistical examination of the association between the FAEE concentrations present in neonatal hair and various clinical characteristics.

It should be noted that Caprara et al. previously published a study that evaluated baseline levels of FAEE in neonatal hair using GC-MS analysis (2005b). This was a pilot study
conducted on 62 infants born to non-alcohol exposed or mild social drinking mothers. The GC-MS method that was used to analyze neonatal hair differed in a number of ways from the GC-MS method that is currently used by our study group. The method involved a different extraction protocol, had only one IS (heptadecanoic acid ethyl ester), used solid-phase extraction rather than headspace solid-phase microextraction and was conducted in chemical ionization mode rather than electron ionization mode. It was determined that this older GC-MS method was subject to false positive results and was later modified.

5.3.2. Method Characteristics and Advantages

To ensure that the LC-MS/MS method is reliable, its results were compared to those derived from GC-MS analysis. The R-values demonstrated a good correlation (≥0.987). The results of the inter and intra-day variability analyses suggest that the method is consistent, given that the %CV did not surpass 12% for both parameters. Similarly, the accuracy and precision experiments were associated with %CVs below 8%, and there was a lack of carryover following the analysis of a sample containing a high concentration of FAEE.

One of the major advantages of this method is that sample analysis takes significantly less time than GC-MS analysis. Whereas GC-MS analysis of a single sample takes approximately 40 minutes run time, total run time is only 6 minutes per sample on LC-MS/MS. Moreover, the use of tandem MS allows for high degree of specificity. This method exhibits good specificity, since the %CVs for retention times did not exceed 0.80% for all analytes and the %CVs for the area ratios were all less than 18%. The extraction efficiencies are adequate, since they were all over 70%. In terms of matrix effects, myristate experiences ion suppression, whereas the three other analytes experience ion enhancement.

5.3.3. Limitations

Ideally neonatal hair would have been used for validation, calibrator and QC samples. Given that neonatal hair was not available in sufficient quantities, child hair was used. Child hair was chosen in lieu of adult hair, because its morphology is comparatively similar to neonatal hair (Flagler et al., 2012). Another limitation is that baseline levels of FAEE are higher in child hair than in neonatal hair; therefore, it was necessary to subtract the area ratio of the
“blank” or non-spiked sample from each point on the calibration curve prior to quantification of samples. However, analysis of EQC adult hair samples revealed that this method for establishing the calibration curve provides reliable results. Additionally, the inter-day variability analysis, which was conducted using the same sources of child hair, but in different proportions than the child hair calibrator pool, produced consistent results using this subtraction method. Due to the lack of non-blank hair, extraction efficiency and matrix effect were evaluated using the deuterated versions of the analytes of interest. However, it is assumed that the deuterated FAEE will exhibit extraction efficiencies and matrix effects similar to the non-deuterated FAEE. Finally, a significant proportion of neonatal hair samples contained levels of FAEE that were at or near the LOQs. Ideally, the analyte concentrations would have been substantially higher than the LOQs in order to have greater confidence in the accuracy and precision of the quantitative results. Despite this limitation, an analyte concentration equal to or above the LOQ was deemed quantifiable and assigned a value, since the pre-specified criteria for bias and imprecision were met based on sensitivity analysis.

5.4. Establishing Baseline Levels of FAEE in Neonatal Hair

5.4.1. FAEE Levels and Their Relation to the Literature

As mentioned in the results section, the mean and median concentrations for each of the four FAEE were approximately the same. The range between the lowest and highest concentration is fairly narrow for each analyte. From a clinical perspective, various combinations of FAEE may be relevant to infer whether there is history of prenatal alcohol use. Palmitate and stearate were combined since they could be quantified in nearly every single sample. These two analytes were also combined with myristate levels, which could be measured in approximately 64% of samples. Among the 177 samples, a total of all four FAEE could only be quantified in 50 samples. This is because oleate could be measured in only 39% (n=69) of neonatal hair samples.

The baseline results published by Caprara et al. involved the analysis of six FAEE rather than four (ethyl laureate and ethyl palmitoleate, in addition to the four FAEE under
investigation in this study) (2005b). Since the Society of Hair Testing 2014 Guidelines recommended the analysis of ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate to determine chronic excessive alcohol consumption in adult hair, these four analytes were chosen to be analyzed in neonatal hair (SoHT, 2014). Unlike the pilot study, which involved the analysis of neonatal hair collected from 62 infants (Caprara et al., 2005b), this study analyzed hair from 200 neonates. The number of individual data points required to establish a reference interval in a healthy/unexposed population is generally considered to be a minimum of 120 (Reed et al., 1971; Horn and Pesce, 2003). Since prenatal alcohol use is subject to under-reporting, stigmatization, and serious ethical and legal implications, an additional 80 samples were collected for a total of 200 samples.

5.4.2. Associations Between Clinical Variables and FAEE Levels, and Their Relation to the Literature

A variety of clinical characteristics were collected not only to characterize the population that participated in the baseline study, but also to determine whether these variables may be associated with levels of FAEE present in the hair. When Chan et al. established the baseline levels of FAEE in meconium, they reported that levels of ethyl myristate were positively related to gestational age (2003). This was explained by the fact that levels of myristic acid increase as pregnancy progresses and peak concentration is achieved in the second trimester, which is maintained throughout the remainder of the pregnancy (Sanjurjo et al., 1993). It was also reported that maternal age was negatively correlated with levels of palmitate. The authors speculated that this association might be due to changes in maternal dietary habits (i.e. increased consumption of olive oil, which is rich in FAEE) (Chan et al., 2003; Pérez-Camino et al., 2002). However, maternal and gestational age were not associated with levels of FAEE in neonatal hair in our study population. Data relating maternal dietary habits were not collected in this study.

Levels of FAEE measured in neonatal hair did not differ on the basis of whether there was low-level drinking in the third trimester of pregnancy. Similarly, Caprara et al. found that levels of FAEE were not significantly different between teetotalers and mild social drinking women (2005b). Together, these findings suggest that FAEE testing in neonatal hair will not
be able to differentiate between teetotalers and women who consume one drink or less per week in the third trimester of pregnancy. However, FAEE testing might be useful for detecting heavy drinking in pregnancy.

Maternal yeasts and bacterial infection can lead to the production of ethanol, and may theoretically lead to increased production of FAEE in the neonate (Jawetz et al., 1982). Data on maternal and neonatal antibiotic use around the time of birth was collected, in addition to presence of maternal infection. Associations between these variables and levels of FAEE in neonatal hair were not found in our study or the previously published meconium baseline study (Chan et al., 2003). Interestingly, the association between levels of palmitate in neonatal hair and the number of neonatal antibiotic treatment days approached significance. The latter variable is likely a surrogate for the presence of a neonatal infection, with an increasing number of treatment days being associated with a more serious and/or chronic infection. It is possible that the presence of infection in the neonate is leading to increased production of ethanol, which is associated with higher levels of FAEE. Presence of neonatal complications associated with increasing levels of various FAEE also approached significance. A couple of neonates were reported as having a fever, which may be associated with infection (Baraff et al., 1993). It is also possible that neonates have higher levels of FAEE due to the presence of a metabolic disorder. It has been proposed that individuals with metabolic disorders, such as diabetes, cirrhosis and hepatitis, may theoretically have higher endogenous levels of ethanol in the body leading to increased formation of FAEE (Chan et al., 2003). This might explain why the association between presence of a medical condition (which includes diabetes) in female patients and total FAEE concentration approached statistical significance. However, previously published studies have not observed this association (Caprara et al., 2005b; Logan and Jones, 2000).

It is also important to consider that there is likely a multitude of physiological, environmental and genetic factors that contribute to the levels of FAEE present in hair. In the clinical context, the number of neonatal antibiotic treatment days, neonatal complications and medical condition in the mother might be factors to consider when interpreting FAEE levels following hair analysis.
5.4.3. Limitations

This study was not specifically powered to examine the association between FAEE levels measured in neonatal hair and the various clinical variables collected in this study. It is likely that this study was underpowered to yield statistically significant results. Moreover, all medical conditions, medications, pregnancy complications and neonatal complications were grouped together within their respective categories. This is not ideal given that there is significant heterogeneity within each of these variables. However, the small numbers of women who had a specific medical condition, took a particular medication or experienced a specific complication did not allow for a thorough statistical examination. This applied to neonatal complications as well. There may also be maternal recall bias of drinking history in the last two trimesters of pregnancy. It should be noted that one of the limits of neonatal hair testing is that it only captures the history of alcohol use in the third trimester of pregnancy (Moller et al., 2010; Gareri and Koren, 2010). However, neonates born to women who drank into their third trimester of pregnancy are likely to be most at-risk for FASD, because women who continue to drink into the third trimester are more likely to have alcohol abuse issues. The risk of FAS is highest among women with alcohol addiction problems (Abel, 1999). Moreover, drinking in the third trimester of pregnancy can affect brain development and the physiological function of various organs. Finally, the utility of FAEE analysis at birth is somewhat limited given that FASD diagnosis does not take place until years later. Ideally, children identified as having been exposed to alcohol prenatally at birth will be flagged to receive timely diagnosis and medical intervention.
Chapter 6
CONCLUSION

6.1. Overview of Findings

The goal of the pharmacogenetic work has been to provide insight into the role of genetic biomarkers that may assist in the management of postpartum pain with opioids. The therapeutic objective is to optimize pain relief in the mother in order to reduce the risk of thromboembolic events and to allow her to care for her neonate, while protecting the mother-child pair from genetically-mediated adverse events following opioid exposure. It was hypothesized that genetic polymorphisms pertinent to the opioid’s pharmacological pathway are linked to analgesic response in the post-cesarean patient and adverse side effects in the mother-child pair. A systematic review of the literature and a nested cohort study were conducted to investigate this hypothesis. The available literature suggests that CYP2D6, OPRM1 A118G, UGT2B7 C802T and ABCB1 G2677AT may contribute to postpartum analgesia and adverse events, or adverse events alone. The pharmacogenetic analyses in the nested cohort study revealed that the OPRM1 A118G and UGT2B7 C802T variants predict codeine consumption in the cohort of post-cesarean patients and within the Caucasian sub-population. Together, these findings support the hypothesis and may assist in personalizing care for patients receiving opioids during the postpartum period, which may serve to reduce adverse outcomes in post-cesarean mothers and their neonates. Moreover, these results shed light on pharmacogenetic factors that may influence opioid pharmacology in opioid-abusing women, which may assist clinicians in dealing with drug-drug interactions, thereby potentially reducing the risk adverse outcomes in this sub-population and in neonates born to this segment of women.

Women who abuse opioids in pregnancy are likely to consume alcohol as well. In order to improve the means of indentifying alcohol use/abuse during pregnancy, an LC-MS/MS method was developed and validated for the analysis of the alcohol biomarkers (FAEE) in neonatal hair. In the majority of cases, confirmation of prenatal alcohol use is required to make an FASD diagnosis. An early diagnosis can facilitate prompt medical intervention, which may serve to improve outcomes in the FASD-affected child. It was
hypothesized that an acceptable method can be developed and validated to establish reference FAEE levels in the hair of neonates exposed to little or no alcohol during gestation. A method which exhibits improved analytical sensitivity relative to existing methods involving the analysis of FAEE in hair was established and validated. This allowed for the establishment of baseline levels of FAEE present in the hair of 177 neonates born to women who consumed one drink or less per week during the third trimester. The results of this work support the hypothesis. Relationships between various population characteristics and FAEE concentrations in neonatal hair were explored. Although there were no statistically significant findings, it was found that the associations between various FAEE concentrations and neonatal antibiotic treatment days, neonatal complications and presence of medical condition in the mother approached significance. It is anticipated that this validated method and the established reference levels could assist in the determination of prenatal alcohol exposure in the immediate postpartum period. Improving identification of ethanol-exposed neonates can increase the rate of diagnosis and allow for a greater proportion of the population to receive medical treatment. This can serve to reduce the risk of developing secondary disabilities.

The results of this thesis may assist in developing the use of genetic polymorphisms as therapeutic biomarkers in the context of postpartum pain management, and the use of FAEE in neonatal hair as substance abuse biomarkers to assist with FASD diagnosis. Implementation of these biomarkers in the immediate postpartum period may serve to reduce the risk of adverse outcomes in mothers and their neonates.

6.2. Future Studies

The role of CYP2D6, UGT2B7 C802T and ABCB1 G2677AT in postpartum analgesia and adverse events in mother and/or infant will still need to be clarified in future studies. A case–control design can be used to determine the role of these polymorphisms by comparing the genetic profiles of patients that report achieving adequate analgesia and/or no side effects in themselves and their neonates to patients that experience suboptimal pain relief and/or adverse events in themselves and their neonates. Future studies should explore the possibility of dose adjusting according to the OPRM1 A118G genotype to determine its clinical
utility, given that the findings of this thesis suggest that it may be a factor contributing to analgesia and side effects among post-cesarean patients. Future pharmacogenetic studies should also aim to have adequately powered randomized control trials. The value of genetically guided treatment can be explored prospectively by randomizing patients to receive ‘care as usual’ or treatment according to genotype. Studies should also be conducted to examine the role of pharmacogenetic markers in the opioid-dependent population.

Future studies will need to evaluate levels of FAEE in neonates born to mothers who drank chronically or heavily in the third trimester of pregnancy, in order to establish a baseline cut-off value at which it can be reasonably ascertained that prenatal alcohol use took place. These studies will also need to consider the interplay between various clinical variables and other factors that may potentially modulate levels of FAEE present in neonatal hair. Finally, future studies should explore whether levels of FAEE in neonatal hair are predictive of an FASD diagnosis. Higher levels of FAEE in the neonate may be associated with more adverse outcomes.
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APPENDIX A: Consent Form for Nested Cohort Study

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
Prospective Recruitment

Study Title:
CYP2D6 Screening for Adverse Drug Reactions to Codeine in Breast Milk – Confirmatory Study

Investigators:

On Site Primary Investigator:
Dr Howard Berger, Dermatologist, St. Michael’s Hospital, Department of Obstetrics and Gynecology, Phone: (416) 867-7460 Ext. 8408

Off-Site Primary Investigator:
Dr Gideon Koren, The Hospital for Sick Children, Department of Clinical Pharmacology
Ivey Chair in Molecular Toxicology, Department of Medicine, University of Western Ontario
Phone: (416) 813-5781

Study Coordinators:
Parvaz Madadi, The Hospital for Sick Children, Department of Clinical Pharmacology
416 813-7654 (x4413)
Shahnaz Akhtar Chaudhry
Sondea van der Vaart
Myla Moretti
Geert W T Jong

(All investigators available: Monday – Friday 9 am – 4 pm)

Introduction:

Before agreeing to take part in this research study, it is important that you read the information in this research consent form. It includes details we think you need to know in order to decide if you wish to take part in the study. If you have any questions, please ask the study doctor or study staff to explain any words you don’t understand before signing this consent form. You will also have the opportunity to ask any additional questions on the day of your delivery. Make sure all your questions have been answered to your satisfaction before signing this document.

All research is voluntary. You may also wish to discuss the study with your family doctor, a family member or close friend.

Background Information:

Women who have a baby by vaginal delivery or c-section are routinely given Tylenol 3 (with codeine) to manage their pain. Codeine is turned into morphine in the body by a specific gene
(CYP2D6). A very small percentage of people have multiple copies of this gene yielding greater than normal morphine levels in the body after they take Tylenol 3; these people are referred to as Ultra rapid-metabolizers.

Morphine passes into the breast milk. Greater than normal levels of morphine can make babies drowsy and lead to morphine toxicity. We believe women and their doctors are usually able to identify morphine toxicity and change a woman’s pain medication before a serious side effect occurs.

**Purpose of Research:**
We believe that knowing whether you were an Ultra rapid-metabolizer would guide your choice of pain medication when breast-feeding. This will lead to reduce incidence of morphine toxicity if you were an Ultra rapid-metabolizer. In order to do this, we will compare women who are tested in advance of receiving pain medication to women who are tested after receiving pain medication.

**Description of Research:**

Once your baby is born, you and your baby will be cared for in the hospital for an average of three days. During this time if you feel pain, you will be given pain medication as standard of care. One of the pain medications is Tylenol 3. This is a multi-centered study. A total of 600 women will be recruited. At St. Michael’s Hospital 300 women were previously recruited into the study after they had given birth to their baby. Currently, we are recruiting women into the study before they are scheduled to have a Caesarian section delivery. St. Joseph’s Hospital in London, Ontario is also recruiting women into the study before they are scheduled to have Caesarian section delivery. At St. Michael’s Hospital, the majority of the participants will receive the standard care with no change to their usual treatment; the only difference will be a saliva sample. It is expected that a small group of women will be identified as codeine ultrarapid metabolizers before their scheduled C-sections, and the doctors of these individuals will be advised to prescribe non-opioid pain medications for postpartum pain relief in these instances. If you agree to participate in this study, we will ask you to give a saliva sample and test whether or not you have multiple copies of the CYP2D6 gene. Also at this time you will be asked to complete a short survey that will take 10 to 20 minutes to complete. You will receive the test results from the Study doctor and you will be given information about the results. These results will be used to guide your postpartum pain therapy. You can opt out of the study at any time. This will not affect your care in the hospital or the care provided to you by your doctor after you leave the hospital.

As a result of participating in is study, once you leave the hospital, we will provide you with a number that is staffed with pediatricians from The Hospital for Sick Children and is available for you: 24 hours/day, 7 days/week while you are taking codeine medication (Tylenol 3) for your pain. If you have any questions about your baby’s alertness or condition, you will be able to speak with a pediatrician and you will be advised as necessary.

If the on-call pediatrician suspects that your baby needs following-up, he/she may direct you to the hospital. At the hospital, if the study physicians suspect that your baby is having problems.
due to codeine, they may ask your permission to take a blood sample from your baby to verify this. You have the right to decline this. Counseling will be provided, and you have the right to decline this as well.

After 3 or 4 days from discharge, a member of the study team will call you to follow-up and see how you and your baby are doing.

You will be given a Patient Medication and Breastfeeding Tracking Sheet and a cost questionnaire to complete with very clear instructions. These forms will be completed over several days and take a total of 10 minutes to complete. You will also be given the same survey you completed at enrollment to complete again, after you have finished your pain medication.

**Breastmilk Collection**

You will be asked to provide a sample of your breast milk at your convenience when you are at home, while still taking a codeine-containing medication. If you agree to participate in this part of the study (if you do not agree, you can still do the other part of the study), the study coordinator will explain to you while you are in hospital, how to collect the milk. A prepaid FedEx package will be provided to you to ship the sample.

**Potential Harms (Injury, Discomforts or Inconveniences):**

Collection of these saliva or breastmilk samples will not affect the care provided to you or your baby.

The collection of saliva and breastmilk carries no known risk to you or your baby.

**Potential Benefits:**

Should you opt to learn of your codeine metabolism status, this information may guide your choice of pain medication in your current and future deliveries. The results from this study may improve our understanding of the best way to treat pain and prevent morphine toxicity in mothers and newborns. We hope that the information obtained in this study will allow us to develop new treatment options for women after cesarean section.

**Protecting Your Health Information:**

All persons associated with this study, including study investigators, coordinators, nurses and delegates (hereby referred to as “study personnel”) are committed to respecting your privacy. No other persons will have access to your personal health information or other identifying personal information without your consent, unless required by law. Any personal health information collected from your medical records, laboratory samples or other information related to you will be coded by study numbers and initials to ensure that persons outside of the study will not be able to identify you. The study personnel are in control of the study code key, which is needed to connect your personal health information to you. Our guidelines include the following:

- All information that identifies you, both paper copy and electronic information, will be kept confidential and stored and locked in a secure place that only the study personnel
will be able to access.
- Electronic files will be stored securely on hospital or institutional networks or securely on any portable electronic devices.
- No information identifying you will be allowed off site in any form. Examples include your hospital or clinic charts, copies of any part of your charts, or notes made from your charts.

It is important to understand that despite these protections being in place, there continues to be the risk of unintentional release of information. The study personnel will protect your records and keep all the information in your study file confidential to the greatest extent possible. The chance that this information will be accidentally released is small.

By signing this form, you are authorizing access to your medical records by the study personnel, and the St. Michael’s Hospital Research Ethics Board. Such access will be used only for the purpose of verifying the authenticity and accuracy of the information collected for the study, without violating your confidentiality to the extent permitted by applicable laws and regulations. Your name will not be used in any publication. None of the research results will be placed in your medical records.

**Participation and Withdrawal:**

Your participation in this study is voluntary. If you do not want to participate in this study, or wish to withdraw at any time, you are free to do so and this will in no way affect your present or future care.

**Potential Cost of Participation and Reimbursement:**

There are no costs associated with participating in this study. You will not be reimbursed for your participation in this study.

**Compensation for Injury:**

If you become ill or are physically injured as a result of participation in this study, medical treatment will be provided to you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this consent form waive your legal rights nor does it relieve the investigators or involved institutions from their legal and professional responsibilities.

**Publication of Results:**

Once the study is complete the information will be summarized and submitted to a medical journal for publication. The outcome of this study may also be presented at conferences, scientific meetings and other public forums. It is important that you are aware that you will not be identified in any of these reports and your confidentiality will be completely maintained.

**Development for Commercial Gain:**

CYP2D6 Screening for Adverse Drug Reactions to Codeine in Breast Milk – Confirmatory Study
Page 4 of 6
REB# 09-038
Version 6 August 26, 2010.
Research carried out on your samples by researchers at the Hospital for Sick Children, or their collaborators, may lead to the development of marketable treatments, devices, new drugs or patentable procedures. By participating in this study you will not benefit directly from any such commercial products that will remain with the Hospital for Sick Children and their research partners.

**Research Ethics Board Contact:**

If you have any questions about your rights as a research participant, you may contact the Chair of the Research Ethics Board, 416-864-6060 ext 2557.

**Further Questions:**

You have been given a copy of this information and consent form. If you have any questions about taking part in this study, you may contact Dr. Gideon Koren (The Hospital for Sick Children) at (416) 813-5781 or Dr. Howard Berger (St. Michael’s Hospital) at (416) 867-7460 Ext. 8408.
Study Title: CYP2D6 Screening for Adverse Drug Reactions to Codeine in Breast Milk – Confirmatory Study

Consent:

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of my right not to participate and the right to withdraw without compromising the quality of my medical care at St. Michael’s Hospital. As well, the potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study.

I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity. I have been given sufficient time to read and understand the above information.

By signing this consent form, I agree to participate in this study.

I hereby consent to participate and will be given a copy of this consent form.

Participant’s Name (Please Print)  Participant’s Signature  Date

Name & Position of Person Obtaining Consent  Signature  Date
APPENDIX B: Questionnaire for Nested Cohort Study

1 General Study Data

Elective C-section date:
Date of discharge:

2 Patient details

Demography
(removes this part from CRF, keep in separate lockable cabinet, only accessible to primary investigators)

Name: ____________________________
Date of birth: _________________ mm/dd/yyyy
Hospital ID number: ____________________________
Patient Address: ____________________________

3 Inclusion/exclusion criteria

Inclusion criteria

<table>
<thead>
<tr>
<th>Question</th>
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<th>No</th>
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<td>Will she have a caesarean section?</td>
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<td>☐</td>
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<tr>
<td>Is informed consent given for the mother?</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>Is informed consent given for the child?</td>
<td>☐</td>
<td>☐</td>
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</table>

If any of the “no” boxes has been marked, the patient is not eligible to participate in this study.

Exclusion criteria

<table>
<thead>
<tr>
<th>Question</th>
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<th>No</th>
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</thead>
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<td>Does she have problems with the English language?</td>
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<td>☐</td>
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<tr>
<td>Will she not be using codeine medication?</td>
<td>☐</td>
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</table>

If any of the “yes” boxes has been marked, the patient is not eligible to participate in this study.
4 Registration

Ethnicity
- Caucasian
- Arabic
- Afro-Caribbean
- Asian
- Hispanic
- Mixed
- Other

Age: _______ years

Weight: _______ kg/lb

Previous C-section: □ Yes □ No  Number: _______

Planning breastfeeding □ Yes □ No

Feeding □ Bottle □ Breastfeeding

Birth Characteristics of Baby

Birth Weight: _______________________
Length: ____________________________
Sex: _______________________________
Gestational Age: ____________________
Other: _____________________________

Gravida/Parity/Term: P_______ G_______ T_______ A_______

Date written consent received: ________________ mm/dd/yyyy
5. Clinical Course during hospital stay

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<th>End date</th>
<th>Intervention</th>
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<td>mm/dd/yy</td>
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6. Drug therapy during hospital stay
(Spinal procedure)

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<th>Note</th>
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<th>Start date (mm/dd/yr)</th>
<th>Start time</th>
<th>Dose</th>
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7. Drug therapy during hospital stay continued
(All codeine medications and morphine equivalents)

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<th>Start date (mm/dd/yr)</th>
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8. Drug therapy during hospital stay
(other than codeine containing Rx)

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<td>Postpartum</td>
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<td>Analgesics, Opioid - (explode)</td>
<td>Peripartum period or Postpartum period Lactation</td>
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<tr>
<td>Polymorphism, Genetic/NT Genomic Structural Variation</td>
<td>NT Drug Terms (non-MeSH) (use these in a keyword search)</td>
<td>NT Milk ejection/Infant (1 to 23 months old)</td>
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<td>Alfentanil</td>
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<td>Levorphanol</td>
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<td>Meperidine</td>
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<td>EMTREE Terms</td>
<td>Meptazinol</td>
<td>Methadone</td>
<td>Methadyl Acetate</td>
<td>Morphine</td>
<td>Nalbuphine</td>
<td>Opiate Alkaloids</td>
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<td>NT acetylator phenotype</td>
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<td>NT pharmacogenomics</td>
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<td>NT toxicogenetics</td>
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<td>amplified fragment length polymorphism</td>
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<td>BT narcotic analgesic agent (explode to include list of drugs)</td>
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<td>BT postnatal care</td>
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<td>NT puerperium (Used for postpartum period)</td>
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<td>postpartum pain</td>
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<td>DNA Polymorphism</td>
<td>NT Newborn Care</td>
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<td>NT Newborn Assessment</td>
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<td>Protein Polymorphism</td>
<td>NT Newborn Intensive Care</td>
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<td>Restriction Fragment Length</td>
<td>NT Newborn Monitoring</td>
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<td>Single Nucleotide Polymorphism</td>
<td>NT Newborn Nursing</td>
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<td>Single Strand Conformation</td>
<td>NT Rooming In (I don't think any of these are relevant or necessary if we use a keyword search for newborn)</td>
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<td>Polymorphism</td>
<td>Cesarean Section</td>
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<td>Personalized Medicine</td>
<td>Breast Feeding</td>
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<td>NT Breast Milk Expression</td>
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<td>Newborn</td>
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APPENDIX D: Quality Assessment Scale for Systematic Review

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation *
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases *
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls *
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) *
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for ____________ (Select the most important factor.) *
   b) study controls for any additional factor *(This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) *
   b) structured interview where blind to case/control status *
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes *
   b) no

3) Non-Response rate
   a) same rate for both groups *
   b) non respondents described
   c) rate different and no designation
NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average ______________ (describe) in the community ∗
   b) somewhat representative of the average ______________ in the community ∗
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ∗
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) ∗
   b) structured interview ∗
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes ∗
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for ______________ (select the most important factor) ∗
   b) study controls for any additional factor ∗ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ∗
   b) record linkage ∗
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ∗
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for ∗
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost ∗
   c) follow up rate < ____ % (select an adequate %) and no description of those lost
   d) no statement
APPENDIX E: Consent Form for Neonatal Hair Study

Consent to Participate in a Research Study

Title of Research Project: Validation of Neonatal hair Analysis and Re-Evaluation of Population Baseline of Meconium Fatty Acid Ethyl Esters to Determine Prenatal Alcohol Exposure

Principal Investigators: Dr. Howard Berger MD; Head, Maternal-Fetal Medicine, St. Michael’s Hospital Assistant Professor, University of Toronto
PH: (416) 864-6060 ext. 2395
bergerh@smh.ca
Availability: Monday to Friday 9-5pm

Joey Gareri, M.Sc.
Laboratory Manager
Motherisk Program, Division of Clinical Pharmacology & Toxicology
Hospital for Sick Children
Project Investigator, Research Institute
PH: (416) 813-5780
joey.gareri@sickkids.ca

Co-Investigators: Marta Baber, B.Sc. (Hons); Graduate Student (PhD candidate)
Motherisk Program, Division of Clinical Pharmacology and Toxicology
The Hospital for Sick Children
Department of Pharmacology and Toxicology
University of Toronto
Supervisor: Dr. Gideon Koren
PH: (416) 813-7709
marta.baber@sickkids.ca

Netta Fulga, M.Sc., Quality Manager, Motherisk Laboratory
Hospital for Sick Children
PH: (416) 813-8216
nettfulga@sickkids.ca

Shinya Ito MD, FRCPC; Division Head, Clinical Pharmacology and Toxicology
The Hospital for Sick Children
Professor, University of Toronto
PH: (416) 813-5776
shinya.ito@sickkids.ca

Population Baseline Assessment of FAEE in Neonatal Hair and Meconium
Version 4.0, May 2015
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Before agreeing to take part in this research study, it is important that you read and understand the information in this research consent form. It includes details we think you need to know in order to decide if you wish to take part in this study. If you have any questions after you read through this form, ask the study coordinator. You should not sign this form until you are sure you understand the information. You may also wish to discuss your participation in this study with your family doctor, a family member, or close friend.

Conflicts of Interest
There are no conflicts of interest between the researchers and the funders of this project. The Principal Investigators, Dr. Howard Berger and Joey Gareri, and the other research team members have no conflict of interest to declare.

Study Sponsor: Canadian Foundation on Fetal Alcohol Research

Purpose of the Research
Why are you being invited to take part in this study?
The Motherisk Program at the Hospital for Sick Children is working in collaboration with St. Michael’s Hospital to develop and validate a new laboratory method to detect fetal exposure to alcohol. You are being invited to participate in this study, because you have recently given birth.

What is the purpose of this study?
The purpose of this study is to evaluate the naturally occurring levels of alcohol metabolites, fatty acid ethyl esters (FAEE), in the hair and meconium (baby’s first stool) of newborns exposed to no alcohol or one drink or less per week during the second and third trimesters of pregnancy.

Requirements of Participation
The study seeks to recruit women who consumed no alcohol or one drink or less per week during the second and third trimesters of pregnancy.

Background and Goals of the Study
FAEE are metabolites of alcohol produced in the body through an enzymatic reaction combining alcohol (i.e. ethanol) and fatty acids. Very low amounts of alcohol naturally occur in the body as a by-product of digestion of certain foods, therefore; very low levels of FAEE are expected to be present even in the absence of any alcohol consumption.

Through a series of animal and human studies, fatty acid ethyl esters (FAEE) in meconium (a baby’s first stool) have been established as promising biomarkers for fetal alcohol exposure. A biomarker is a measurable characteristic that is linked to particular biological condition or state. Determining fetal alcohol exposure in at-risk infants is critically important in order to facilitate early diagnosis of Fetal Alcohol Spectrum Disorder (FASD) and mobilize support services early in the child’s development.

While meconium has been shown to be very useful in this regard, it is only available for a couple of days after birth. Conversely, the hair a newborn is born with can be collected anytime in the first several weeks of life and is widely used to assess for prenatal exposure to drugs of abuse, such as cocaine and methamphetamine.

This study aims to establish the normal (i.e. “baseline”) levels of FAEE occurring in non-alcohol exposed infant hair by testing the hair of newborns born to mothers consuming no alcohol or low levels of alcohol (less than or equal to 1 drink/week on average) during the last two trimesters of pregnancy. By testing non-exposed newborn hair and comparing the FAEE levels to those found in hair from newborns with known exposures (these will be collected from our clinical laboratory population); we can determine a

Population Baseline Assessment of FAEE in Neonatal Hair and Meconium
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“cut-off” level of FAEE that can effectively differentiate between alcohol-exposed and non-exposed newborns.

In addition to collecting hair, we are also re-evaluating baseline FAEE concentrations in the meconium of non-exposed newborns using newer analytical techniques than those used in the original study examining baseline FAEE concentrations, published in 2003.

**Description of the Research**

Approximately 210 women will be recruited through the Motherisk Counseling Service at the Hospital for Children and the Labour and Delivery Unit at St. Michael’s Hospital.

Meconium samples must be collected within twenty-four hours after birth. Meconium samples are scraped out of soiled diapers. Hair samples will be collected from newborns within twenty-eight days after birth. If immediate hair sample collection is not possible, you have the option of attending the Motherisk maternal-fetal clinic for neonatal hair sample collection within twenty-eight days after the birth of your child or to have sample collection conducted at your home, at your convenience, by the study coordinator. Hair samples are collected by cutting two 10 milligram hair samples from the child’s scalp in as inconspicuous an area as possible. Here is a visual representation of what 10 milligrams of hair looks like:

![Image of hair samples]

We ask that you consent to collection of both hair and meconium from your newborn; however, you are free to choose to contribute either hair or meconium. In order to participate in the study, we will also require you to complete a ten minute questionnaire providing the following information: frequency of alcohol beverage consumption in the last two trimesters of pregnancy, maternal age, maternal and neonatal infection around time of birth, use of antibiotics around time of delivery (specific date of administration), maternal health conditions (such as diabetes), pregnancy complications, date and time of birth, gestational age at birth and time & date of first bath. You will not be required to answer all questions if you do not wish to, however, this may prevent you from being eligible to participate in the study. Any therapy that you were receiving prior to enrollment in the study will not altered or discontinued as a result of participation in the study.
**Potential Harms (Injury, Discomforts or Inconvenience)**

We know of no harm that taking part in this study could cause you. Meconium and hair sample collection produce no discomfort or pain to your newborn. There may be a potential aesthetic concern of having a clipping of your newborn’s hair taken. If you choose to attend the Motherisk maternal-fetal clinic for neonatal hair sample collection, the time commitment required to participate and travel to the Hospital for Sick Children may be an inconvenience.

**Potential Benefits**

You and your child will not benefit directly from participating in this study. However, this study will contribute to improving our ability to detect prenatal alcohol exposure and identify vulnerable, at-risk children who require specialized health services. Successfully identified and treated individuals with FASD have significantly lower risk of dependent living, incarceration, addiction issues, and future social services involvement. Addressing these outcomes associated with FASD through early identification in the future substantial potential to significantly reduce burdens on the child welfare and justice systems.

**Protecting Your Health Information**

We will respect your privacy. No information about who your child is will be given to anyone or be published without your permission, unless required by law. For example, the law could make us give information if a child has been abused, if your child has an illness that could spread to others, if you or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers. Alcohol consumption is self-reported in this study. Should you self-disclose high levels of alcohol consumption during the recruitment process, or if your child’s meconium sample shows signs of high alcohol use, we will advise the physician in charge of your care that your child is at risk for FASD and that you may have an undiagnosed alcohol use disorder. It will be according to your physician’s discretion as to whether you are displaying any functional impairments that place the child at immediate risk of abuse or neglect, which would necessitate referral to Children’s Aid Society. Your physician may also choose to refer you to the Motherisk Clinic at the Hospital for Sick Children or the FASD Diagnostic Clinic at St. Michael’s Hospital for post-natal counseling and early developmental follow-up for your child.

Only selected members of the research team will have access to the study data. The data will not be provided back to the study sponsor. Following completion of the research study, the data will be kept 5 years following publication and subsequently destroyed according to Sick Kids policy. Questionnaires containing health information of participating subjects will be physically held by the study coordinator and directly transported to the Hospital for Sick Children Research Institute (PGCRL). Questionnaires will be immediately stored in a locked filing cabinet in a room with a locked door. The PGCRL is a secure building. Only authorized staff is permitted entry above the 3rd floor. The questionnaire and samples will be assigned a unique identifier to link them. Linkages cannot occur in the absence of the ID key, which will link maternal name and child’s date and time of birth to the unique identifier. The ID key will be kept in a secure location by the study coordinator on a password-protected SickKids Hard Drive. Informed consent forms bearing maternal name and signatures will be kept in a locked cabinet separate from the questionnaires, which will be stored in a different locked cabinet. All identifying information will be purged following completion of analysis. The data produced from this study will be stored in a secure, locked location. All computer files will be kept locked with restricted access passwords known only to the investigators. Data stored electronically will be saved on a password protected internal server that has firewalls and security measures in place. Data saved to mobile devices will be password protected and encrypted. The study coordinator will physically transfer neonatal hair and meconium samples from St. Michael’s Hospital to the PGCRL. Neonatal hair samples will be transported in a backpack that is secured with a combination lock and subsequently stored in a locked filing cabinet in a room with a locked door on the 10th floor of the PGCRL. Meconium samples will be stored frozen at -80 degrees on
the 10th floor of the PGCRL where sample analysis will be taking place. Following sample analysis, excess neonatal hair and meconium samples will be stored for 5 years following publication and then destroyed according to standard laboratory policies and procedures. Biological samples and data derived from their analysis are solely intended for research purposes.

Personal health information and hair/meconium samples are being collected solely for the proposed research outlined above. The investigators intend to publish and present the results at various conferences and scientific rounds. Study results will not reveal you or your child’s identity at any point in time. Experience in similar studies indicates that the greatest risk in this study to you is the unintentional release of information from your health records. The study coordinator will protect your records and keep confidential all the information in your study file, including your name, address and telephone number. The chance that this information will accidentally be given to someone else is small. Sick Kids Clinical Research Monitors, employees of research granting foundation (Canadian Foundation for Fetal Alcohol Research), regulator of the study or the Research Ethics Board at St. Michael’s Hospital and the Hospital for Sick Children may see your study file to check on the study. By signing this consent form, you agree to let these people look at your records. You may be contacted by a representative of the Research Ethics Board to ask you questions about your experience with the recruitment and consent process or regarding your experience in the study, with a view to assuring and improving the quality of those processes.

Study Results
Once the study is complete, a summary of findings will be posted on the Motherisk Program website (www.motherisk.org). If you have further questions or concerns you may contact Joey Gareri at joey.gareri@sickkids.ca.

Potential Costs of Participation and Reimbursement to the Participant
If you choose to attend the Motherisk maternal-fetal clinic for neonatal hair sample collection on a later date, we will reimburse you for all your reasonable out of pocket expenses for being in this study e.g., meals, babysitters, parking and getting you to and from the Hospital for Sick Children. If you stop taking part in the study, we will pay you for your expenses for taking part in the study up until that point. Please submit receipt for out of pocket expenses to the principal investigator for reimbursement, Joey Gareri.

Compensation for Injury
If you suffer a physical injury from participating in this study, medical care will be provided to you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor release the study investigators, sponsors or involved institutions from their legal and professional responsibilities.

Participation and Withdrawal
Participation in any research study is voluntary. If you choose not to participate, you and your family will continue to have access to usual care at St. Michael’s Hospital. If you decide to participate in this study you can change you mind without giving a reason, and you may withdraw from the study at any time without any effect on the care you and your family will receive at St. Michael’s Hospital. If you choose to withdraw from the study, data compiled up to that point will not be included in the study. You will not be required to answer all questions on the questionnaire if you do not wish to, however skipping questions #1,3 and 4 will prevent you from participating in the study.

Research Ethics Board Contact
If you have questions about your rights as a subject in a study or injuries during a study, please call Dr. David Mazer, Chair of the St. Michael’s Hospital Research Ethics Board at (416) 864-6060 x2557 during regular business hours.

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Study Contact
If you require further information or have problems concerning the study, please contact Marta Baber, Study Coordinator at (416) 230-8377 during regular business hours. For more information on the scientific aspects of the research, please contact Dr. Howard Berger, Principal Investigator at (416) 864-6060 ext. 2395 during regular business hours.
Signature Page

Participant Information and Consent Form (Adult)

*Population Baseline Assessment of Fatty Acid Ethyl Esters (FAEE) in Neonatal Hair and Meconium*

Principal Investigator: Dr. Howard Berger, (416) 864-6060 ext. 2395

The research study has been explained to me, and my questions have been answered to my satisfaction. I have been informed of the alternatives to participation in this study. I have the right not to participate and the right to withdraw without affecting the quality of medical care at St. Michael’s Hospital for me and for other members of my family. As well, the potential harms and benefits (if any) of participating in this research study have been explained to me.

I have been told that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional responsibilities. I know that I may ask now, or in the future, any questions I have about the study. I have been told that records relating to me and my care will be kept confidential and that no information will be disclosed without my permission unless required by law. I have been given sufficient time to read the above information.

I agree, or consent, to the collection of my child’s (please check the relevant box):

- [ ] Hair and Meconium
- [ ] Meconium only
- [ ] Hair only

I consent to participate. I have been told I will be given a signed copy of this consent form.

__________________________________________  ______________________________________
Participant’s Name (please print)                  Participant’s Signature and Date

I, the undersigned, have fully explained the study to the above participant.

__________________________________________  ______________________________________
Name of Person Obtaining Informed Consent          Signature of Person Obtaining Informed Consent and Date

Population Baseline Assessment of FAEE in Neonatal Hair and Meconium
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APPENDIX F: Questionnaire for Neonatal Hair Study

Study Participant Questionnaire / Data Collection Sheet

Unique Identifier

1. Gestational Age at Birth

2. Date of First Bath Time of First Bath

3a. Estimated Number of Alcohol Drinks during Second Trimester
3b. Estimated Drinks per week during Second Trimester
3c. Estimated Drinks per month during Second Trimester

4a. Estimated Number of Alcohol Drinks during Third Trimester
4b. Estimated Drinks per week during Third Trimester
4c. Estimated Drinks per month during Third Trimester

5. Maternal Age

6a. Maternal Bacterial Infection around time of birth: Yes No
   - Strep B-positive? Yes No
   - Other (please describe):

If yes,
6b. Maternal antibiotics around time of birth? If so:
   - Name of medication(s):
   - Date of starting treatment:
   - Date of last dose (if available):

7. Chronic Maternal Disease (e.g. Diabetes, Asthma, Hypertension, etc.):

7b. If so, list any medications taken during pregnancy:

8. Pregnancy Complications:

9a. Neonatal Bacterial Infection around time of birth: Yes No
   - If “Yes”, please describe:

If yes,
9b. Neonatal antibiotics around time of birth? If so:
   - Name of medication(s):
   - Date of starting treatment:
   - Date of last dose (if available):

10. Child’s Date of Birth Time of Birth

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APPENDIX G: Research Ethics Board Approvals

Ethics approval for codeine in breastfeeding

Office of Research Ethics
The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 5C1
Telephone: (519) 888-3000 Fax: (519) 888-2488 Email: ethics@uwo.ca
Website: www.uwo.ca/researchethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Koren
Review Number: 15558
Review Date: July 28, 2009
Revision Number: 1
Revision Date: July 28, 2009

Protocol Title: CYP2D6 screening for Adverse Drug Reactions to Codeine in Breast Milk
Department and Institution: Paediatrics, University of Western Ontario
Sponsor: CIHR-Canadian Institute of Health Research

Ethics Approval Date: July 28, 2009
Expiry Date: December 31, 2012


Documents Received for Information:

This is to notify you that the University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Guidelines and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only minor administrative aspects of the study (e.g. change of contact, telephone number). Expected reviews of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent document.

Investigators must promptly report to the HSREB:
1) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study,
2) all adverse and unexpected experiences or events that are both serious and unexpected;
3) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/ adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in decisions related to any vote on such studies when they are presented in the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

This is an official document. Please retain the original in your files.
September 29, 2016

Dr. Howard Berger,
Department of Obstetrics and Gynaecology,
St Michael’s Hospital

Dear Dr. Berger,

Re: REB# 09-038 - CYP2D6 Screening for Adverse Drug Reactions to Codeine in Breast Milk - Confirmatory Study

REB APPROVAL:  

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<th>Original Approval Date</th>
<th>Annual/Interval Review Date</th>
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<tr>
<td>October 08, 2009</td>
<td>October 08, 2017</td>
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Thank you for your communications dated August 15, 2016 requesting an annual review and approval regarding the above named study.

This letter will serve as an extension of the St. Michael’s Hospital (SMH) Research Ethics Board (REB) approval for the study for a period of 12 months effective from October 08, 2016 – October 08, 2017. Continuation beyond that date will require further review of REB approval.

The deliberation, review or approval of this submission did not include a Research Ethics Board member involved with this study.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

The St. Michael’s Hospital (SMH) Research Ethics Board (REB) operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans, the Ontario Personal Health Information Protection Act, 2004, and ICH Good Clinical Practice Consolidated Guideline E6, Health Canada Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Product Regulations, and the Medical Devices regulations. Furthermore, all investigational drug trials at SMH are conducted by Qualified Investigators (as defined in the latter document).

Good luck with your investigations.

With best wishes

[Signatures]

Dr. David Mazer  
Chair, Research Ethics Board

Dr. Philip Berger  
Vice Chair, Research Ethics Board

Dr. Brenda McDowell  
Vice Chair, Research Ethics Board
Research Ethics Board (REB)
Renewal Approval Letter

2016-07-18

Dr. Shinya Ito
Clinical Pharmacology & Toxicology

REB number: 1000045525

Study title: Validation of Neonatal Hair Analysis and Re-Evaluation of Population Baseline of Meconium Fatty Acid Ethyl Esters to Determine Prenatal Alcohol Exposure
Date of initial study approval: 2014-07-16
Study expiry date: 2017-07-16

Thank you for your renewal application requesting a renewal and approval of the above named study.

This letter will serve as an extension of the SickKids Research Ethics Board (REB) approval for the study. This renewal was approved by the REB via delegated review (not by Full Board review). This approval is effective from 2016-07-16 to 2017-07-16. Continuation beyond that date will require further review of REB approval.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

____________________
Rose Gaiterio, RN, BScN, MSN
REB Vice-Chair

555 University Avenue, Toronto, ON M5G 1X8
Tel: (416) 813-8279 Fax: (416) 813-6515

The SickKids REB operates in compliance with the Tri-Council Policy Statement; ICH Guideline for Good Clinical Practice E6(R1); Ontario Personal Health Information Protection Act (2004); Part C Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations and the Medical Devices Regulations of Health Canada. The approval and the views of the REB have been documented in writing. The REB has reviewed and approved the clinical trial protocol and informed consent form for the trial. All investigational drug trials at SickKids are conducted by qualified investigators.

Furthermore, members of the Research Ethics Board who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.
May 13, 2016

Dr. Howard Berger,
Department of Obstetrics and Gynaecology,
St Michael's Hospital

Dear Dr. Berger,

Re: REB# 14-064 - Validation of Neonatal Hair Analysis and Re-Evaluation of Population Baseline of Meconium Fatty Acid Ethyl Esters to Determine Prenatal Alcohol Exposure

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Thank you for your communications dated April 26, 2016 requesting an annual review and approval regarding the above named study.

This letter will serve as an extension of the St. Michael's Hospital (SMH) Research Ethics Board (REB) approval for the study for a period of 12 months effective from June 20, 2016 – June 20, 2017. Continuation beyond that date will require further review of REB approval.

The deliberation, review or approval of this submission did not include a Research Ethics Board member involved with this study.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

The St. Michael's Hospital (SMH) Research Ethics Board (REB) operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans, the Ontario Personal Health Information Protection Act, 2004, and ICH Good Clinical Practice Consolidated Guideline E6, Health Canada Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Product Regulations, and the Medical Devices regulations. Furthermore, all investigational drug trials at SMH are conducted by Qualified Investigators (as defined in the latter document).

Good luck with your investigations.

With best wishes

[Signatures]

Dr. David Mazer
Chair, Research Ethics Board

Dr. Philip Berger
Vice Chair, Research Ethics Board

Dr. Brenda McDowell
Vice Chair, Research Ethics Board
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George Gibbons <g.gibbons@future-science-group.com>
Mon 8/15/2016 11:51 AM
To Marta Baber <marta.baber@mailutoronto.ca>

Dear Marta,

I am happy to grant that permission.

All the best,

George Gibbons
Sales Support Administrator
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