Risk Factors Associated With Opioid-Induced Toxicity: Ontogeny, Pharmacogenetics, and Drug Interactions

By

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

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Doctor of Philosophy

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Abstract

Opioids are an important class of drugs used for the treatment of pain. The analgesic response and opioid-induced toxicity vary widely among patients. Of serious concern is the use of opioids in susceptible populations, such as infants and their mothers. The overall objective of this thesis was to investigate the contribution of ontogeny, pharmacogenetics and drug interactions in predicting opioid-induced toxicity. This objective was addressed in four separate studies. The first study assessing whether maternal clinical factors are predictive of neonatal central nervous system (CNS) depression, revealed that the replacement of codeine by oxycodone was not safe for the mother-infant pair during breastfeeding and factors such as duration of opioid use, opioid dose, and maternal CNS depression were predictors of neonatal CNS depression. In the second study assessing whether maternal genetic variants in genes involved in the metabolism and response of oxycodone were predictive of neonatal CNS depression found that the interplay between maternal genetic and clinical factors predicted maternal and neonatal CNS depression. In the third study, the ontogeny of P-glycoprotein in the developing human blood brain barrier was assessed and found to be low in newborn infants and increased remarkably with postnatal maturation, suggesting that newborn infants have heightened sensitivity to the
central effects of opioids. Lastly, the role of drug interactions in deaths attributed to codeine were investigated and revealed that the combination use of codeine with other centrally acting drugs was more toxic than codeine alone. Thus, these combinations should be avoided or at least closely monitored. Overall, the results of this research suggest that no single risk factor is associated with opioid-induced toxicity but it is the interplay among clinical factors, genetic polymorphisms, ontogeny of drug-metabolizing enzymes and relevant drug transporters, and drug interactions that will predict opioid-induced toxicity. Understanding the contribution of all these factors will allow for safer prescribing of opioids.
Acknowledgments

“If it were not for the great variability among individuals, medicine might as well be a science and not an art.”
- Sir William Osler, 1892

I would like to thank the following individuals for their guidance, support and contributions during the course of my graduate studies.

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<tbody>
<tr>
<td>6-MAM</td>
<td>6-monoacetylmorphine</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>ABCB1</td>
<td>ATP-binding cassette B1 gene</td>
</tr>
<tr>
<td>ADR</td>
<td>Adverse drug reaction</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the plasma drug concentration versus time curve</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>BCEC</td>
<td>Brain capillary endothelial cell</td>
</tr>
<tr>
<td>BCRP</td>
<td>Breast cancer resistance protein</td>
</tr>
<tr>
<td>BCSFB</td>
<td>Blood-cerebrospinal fluid barrier</td>
</tr>
<tr>
<td>C6G</td>
<td>Codeine-6-glucuronide</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol O-methyltransferase</td>
</tr>
<tr>
<td>CPIC</td>
<td>Clinical Pharmacogenetics Implementation Consortium</td>
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<tr>
<td>CRD</td>
<td>Codeine-related deaths</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CYP</td>
<td>Cytochrome P450</td>
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<tr>
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<td>Cytochrome P450 3A5</td>
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<td>DAMGO</td>
<td>D-Ala2, N-MePhe4, Gly-ol]-enkephalin</td>
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<td>DAWN</td>
<td>Drug Abuse Warning Network</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>Emergency department</td>
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<td>EM</td>
<td>Extensive metabolizer</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>G-protein</td>
<td>Guanine nucleotide-binding protein</td>
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<td>GABA</td>
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<td>GC-MS</td>
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<tr>
<td>GCPR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>GIRK</td>
<td>G protein-coupled inwardly-rectifying potassium channels</td>
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<tr>
<td>HEK293</td>
<td>Human embryonic kidney 293</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>IM</td>
<td>Intermediate metabolizer</td>
</tr>
<tr>
<td>IQR</td>
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<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography- mass spectrometry/ mass spectrometry</td>
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<tr>
<td>LOD</td>
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</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
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<tr>
<td>M/C</td>
<td>Morphine-to-codeine</td>
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<td>M1</td>
<td>O-desmethylnaloxone</td>
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<td>M3G</td>
<td>Morphine-3-glucuronide</td>
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<tr>
<td>M6G</td>
<td>Morphine-6-glucuronide</td>
</tr>
<tr>
<td>MAF</td>
<td>Minor allele frequency</td>
</tr>
<tr>
<td>MOR</td>
<td>Mu opioid receptor</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MRP</td>
<td>Multidrug resistance-associated protein</td>
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<tr>
<td>NAS</td>
<td>Neonatal abstinence syndrome</td>
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<tr>
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<td>Office of the Chief Coroner of Ontario</td>
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<tr>
<td>OPRM1</td>
<td>Opioid receptor mu 1</td>
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<td>OR</td>
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<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
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<tr>
<td>PAG</td>
<td>Periaqueductal grey matter</td>
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<td>-------------</td>
</tr>
<tr>
<td>PBPK</td>
<td>Physiology-based pharmacokinetic</td>
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<tr>
<td>PM</td>
<td>Poor metabolizer</td>
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<td>POMC</td>
<td>Pro-opiomelanocortin</td>
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<tr>
<td>Q6h</td>
<td>Every 6 hours</td>
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<tr>
<td>RID</td>
<td>Relative infant dose</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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</tr>
<tr>
<td>UGT2B7</td>
<td>UDP-glucuronosyltransferase 2B7</td>
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<tr>
<td>UM</td>
<td>Ultra-rapid metabolizer</td>
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Chapter 1 General Introduction

1 Review of the Literature

1.1 Pain Management Using Opioids

1.1.1 Pain Pathway

Pain is a complex sensory and emotional experience that is required for survival. The transmission of a noxious stimulus is automatic and occurs at multiple brain levels. Nociceptors located in the tissues are activated following a painful stimulus, which converts the stimulus into an electrical signal that is relayed to the central nervous system (CNS). The nociceptive neurons are free nerve endings of unmyelinated and finely myelinated neurons that form synapses with the dorsal horn of the spinal cord. The dorsal horn is comprised of physiologically distinct laminae (or layers) that have discrete functions related to pain processing. These nociceptive neurons use nociceptive neurotransmitters such as substance P, acetylcholine, and noradrenaline to relay the pain signal to higher brain centers. From the dorsal horn, nociceptor neurons ascend in the spinothalamic and spinoreticular tracts of the spinal cord to the medulla. Neurons from the medulla synapse onto neurons in the thalamus. After processing the sensory information, it is relayed to the cerebral cortex, hypothalamus, and limbic system, which mediate reflexes and integrated responses (fear, conscious realization of pain, memory and suffering) related to nociceptive impulses.

There are descending pathways responsible for modulating the sensation of pain. The periaqueductal grey matter (PAG) in the midbrain is an important center for the descending control of pain. The PAG receives input from the cerebral cortex, hypothalamus, thalamus and limbic system and carries the signal back to the medulla. Axons from the medulla release nociceptive inhibitory chemical mediators such as endogenous opioids (endorphin, enkephalin, dynorphin) onto dorsal horn to inhibit pain transmission.

1.1.2 Mechanism of Action of Opioids

There are many classes of drugs used for pain management. According to the World Health Organization (WHO), mild to moderate pain should be first treated using non-opioid
medications such as acetaminophen, ibuprofen or aspirin (World Health Organization (WHO), 2014). If pain persists and/or its intensity increases, a weak opioid should be introduced. However, the mainstay for severe pain management is with the use of strong opioids. The pharmacodynamic effects of opioids such as analgesia, sedation, euphoria, respiratory depression, reward and dependence are mediated by binding to opioid receptors distributed throughout the central and peripheral nervous systems (Table 1.1) (Goldstein and Naidu, 1989).

Table 1.1 Opioid receptors involved in mediating some of the pharmacodynamics effects of opioids

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<th>Actions</th>
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</thead>
<tbody>
<tr>
<td>µ</td>
<td>Analgesia&lt;br&gt;Respiratory Depression&lt;br&gt;Euphoria&lt;br&gt;Dependence</td>
</tr>
<tr>
<td>δ</td>
<td>Spinal analgesia&lt;br&gt;Sedation&lt;br&gt;Miosis</td>
</tr>
<tr>
<td>K</td>
<td>Analgesia&lt;br&gt;Respiratory depression&lt;br&gt;Euphoria&lt;br&gt;Constipation&lt;br&gt;Stress response to pain&lt;br&gt;Immunomodulation</td>
</tr>
</tbody>
</table>
Figure 1.1 Molecular mechanism of opioids.
Opioids bind on to opioid receptors located on the presynaptic and postsynaptic neurons and inhibit the release of neurotransmitter, decreasing the probability of the generation of an action potential.

Abbreviation: AC, adenylate cyclase; GIRK, G protein-coupled inwardly-rectifying potassium channel (Johnson et al., 1994; Pasternak and Pan, 2013; Smart et al., 1995).

Three types of opioid receptors, mu (μ), delta (δ) and kappa (κ) have been identified. All opioid receptors are G-protein coupled receptors (GPCR), and have a same mechanism of action when bound to endogenous or exogenous opioids. The analgesic effect of the mu-opioid receptor (MOR) has been thoroughly investigated (Pasternak and Pan, 2013). MOR is coupled via the inhibitory G-protein, G_{al}/G_{oi} to a variety of downstream effectors. When the opioid agonist binds to the MOR, it promotes GDP/GTP exchange and dissociation of heterotrimeric guanine nucleotide-binding protein (G-protein) (Figure 1.1). The G-protein generates two transduction molecules, G_{al}/G_{oi} and βγ subunits. The G_{al}/G_{oi} inhibits adenylate cyclase activity,
which decreases cAMP levels in the neurons (Johnson et al., 1994). Normally neurotransmitter release from the presynaptic neuron is initiated by depolarization via the flow of calcium ions (Ca\(^{2+}\)) through the voltage-gated calcium channels. The βy subunit interacts with the voltage-gated calcium channels and decreases the influx of Ca\(^{2+}\) in the presynaptic neuron. This reduces the release of neurotransmitters (glutamate and substance P) from the presynaptic nerve terminal and thereby decreases neuronal excitability. The βy subunit increases K\(^+\) influx via G protein-coupled inwardly-rectifying potassium channels (GIHKs) resulting in hyperpolarization of the postsynaptic neuronal membrane (Smart et al., 1995). Thus, this shortens the repolarization time and the duration of the action potential leading to inhibition of pain transmission.

1.1.3 Opioid Classification

Several classification systems are available to describe opioids. They can be classified as endogenous, naturally occurring (opiates), semi-synthetic and synthetic opioids based on the source (Table 1.2).

Table 1.2 Classification of opioids based on source linked with receptor affinity

<table>
<thead>
<tr>
<th></th>
<th>Opioid</th>
<th>Receptor Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous</td>
<td>Endorphin</td>
<td>μ &gt;&gt;&gt; δ &gt; κ agonist</td>
</tr>
<tr>
<td></td>
<td>Enkephalin</td>
<td>δ agonist</td>
</tr>
<tr>
<td></td>
<td>Dynorphin</td>
<td>κ agonist</td>
</tr>
<tr>
<td>Opiate</td>
<td>Morphine</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Codeine</td>
<td>Prodrug</td>
</tr>
<tr>
<td>Semi-synthetic</td>
<td>Oxycodone</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Oxymorphone</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Hydrocodone</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Hydromorphone</td>
<td>μ agonist</td>
</tr>
<tr>
<td>Synthetic</td>
<td>Fentanyl</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Methadone</td>
<td>μ agonist</td>
</tr>
<tr>
<td></td>
<td>Tramadol</td>
<td>μ, δ, κ agonist + non-opioid</td>
</tr>
</tbody>
</table>
**Endogenous opioids:** Endogenous opioids are produced by neurons located throughout the central and peripheral nervous system. Three distinct opioid neuropeptides have been identified which are encoded by three different genes: pro-opiomelanocortin (POMC), proenkephalin and prodynorphin (Holden et al., 2005). These large precursors are cleaved to generate the mature neuropeptide endorphin, enkephalin and dynorphin acting on specific opioid receptors, μ, δ, κ receptor, respectively.

**Opiates:** Both morphine and codeine are isolated at high levels from the opium poppy *Papaver somniferum*. The opium plant intrinsically demethylates codeine to form morphine. These opiates are used as a precursor in the synthesis of many opioid derivatives used in the clinical setting. Codeine is a prodrug and requires the conversion into morphine by the cytochrome P450 (CYP) 2D6 enzyme (discussed in further detail in Section 1.1.4.2) to elicit its analgesic effect. Morphine is a potent μ agonist that is commonly used for the treatment of severe pain.

**Semi-synthetic Opioids:** Semi-synthetic opioids (oxycodone, oxymorphone, hydrocodone and hydromorphone) are derivatives of morphine. They are formed having one or several changes made to the chemical structure of morphine. For example, reduction of a double bond on the benzene ring of morphine forms hydromorphone. Thebaine, a minor constituent of the opium poppy, has a similar chemical structure to both morphine and codeine. It does not exhibit any analgesic effect but is used as a precursor for oxycodone and oxymorphone synthesis.

**Synthetic Opioids:** Synthetic opioids (fentanyl, methadone and tramadol) are completely man made and not chemically related to opiates. They are synthesized by a number of drug companies in hope to be used as analgesics with greater receptor selectivity with minimal adverse effects.

1.1.4  Pharmacokinetics of Opioids

1.1.4.1  Absorption and Distribution

All opioids have a similar chemical structure: a basic amino group of different length, an aromatic moiety, and a basic center (Pasternak and Pan, 2013). However, they differ in
numerous pharmacokinetic properties, which influence their absorption, distribution, elimination and onset of action. Opioids are available in different preparations depending on their physiochemical properties and indications. When codeine is ingested orally, it is well absorbed from the gastrointestinal tract (90% bioavailability) and peak plasma concentration is obtained after 60 minutes (Purdue Pharma Std., 2013). Since the site of action for codeine is in the brain, it must cross the blood brain barrier (BBB). It crosses the BBB with greater ease compared to the less lipophilic metabolite, morphine.

Following oral oxycodone administration, the peak oxycodone concentration is reached within a few hours. It has a higher bioavailability than to morphine, ranging from 60 to 90% (Lugo and Kern, 2004). Once it is absorbed in the gastrointestinal tract, it is distributed to the skeletal muscles, liver, lung, spleen and brain (Purdue Pharma Std., 2009).

1.1.4.2 Metabolism and Excretion

All opioids are metabolized in the liver, mainly by the cytochrome P450 (CYP) system and to a lesser extent by the UDP-glucuronosyltransferases (UGT) (Table 1.3). Metabolism influences an individual’s efficacy and tolerability to opioids. Factors such as genetic polymorphisms, drug-interactions and diseases (mainly liver or kidney diseases) can influence the rate and pathways of opioid metabolism and excretion. Opioid metabolism may produce both inactive and active metabolites. Usually the active metabolites are more potent (have a higher affinity for the MOR) than the parent compound, which may have some clinical utility but can also be associated with toxicity (Volpe et al., 2011).
Table 1.3 CYPs and UGTs involved in the metabolism of selected opioids

<table>
<thead>
<tr>
<th>Opioid</th>
<th>Phase I Enzyme</th>
<th>Phase II Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP2D6</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Codeine is a weak opioid with low affinity for the MOR (Volpe et al., 2011). Approximately 50 to 70% of codeine is glucuronidated in the liver by the UDP-glucuronosyltransferase 2B7 (UGT2B7) to form the major metabolite, codeine-6-glucuronide (C6G) (Figure 1.2) (Coffman et al., 1997). Cytochrome P450 3A4 (CYP3A4) is responsible for N-demethylation of approximately 10 to 15% of codeine to norcodeine (Caraco et al., 1996). The highly polymorphic enzyme, cytochrome P450 2D6 (CYP2D6) is responsible for O-demethylating between 0 to 15% of codeine into the active metabolite, morphine (Chen et al., 1991). The influence of genetic polymorphisms on codeine efficacy and toxicity is further discussed in Section 1.2.

A total of 60% of morphine is glucuronidated into morphine-3-glucuronide (M3G) while 5-10% is converted into morphine-6-glucuronide (M6G) via UGT2B7 (Ohno et al., 2008). M6G is pharmacologically active with two to four-fold greater affinity for the MOR in rats and humans compared to morphine (Wittwer and Kern, 2006). UGT1A1 may have a minor role in the conversion of morphine into M3G and M6G (Holthe et al., 2002). The free codeine and codeine
glucuronides (70%) and to a lesser extent free morphine and morphine glucuronides (10%) are excreted by the kidneys. Patients with renal impairment may have reduced morphine and glucuronide clearance, which has been associated with serious adverse effects, including respiratory depression, sedation, nausea and vomiting (Angst et al., 2000; Dubs et al., 1999; Hagen et al., 1991).

CYP2D6 and UGTs are expressed in the brain (Nagano et al., 2000; Wahlstrom et al., 1988). Codeine may cross into the brain and be metabolized into morphine (Siegle et al., 2001). Furthermore, the analgesic effect of codeine/morphine may be enhanced by cerebral enzymatic metabolism of morphine into M6G (Nagano et al., 2000). When the rat brain homogenate was incubated with morphine, M3G and M6G were detected, suggesting that the brain was capable of forming the glucuronide metabolites (Nagano et al., 2000). However, these levels were much lower compared to the liver (Nagano et al., 2000).

![Figure 1.2 Metabolism of codeine in the liver.](image)

Abbreviations: CYP3A4, cytochrome P450 3A4; CYP2D6, cytochrome P450 2D6, UGT2B7, UDP-glucuronosyltransferase 2B7; UGT1A1, UDP-glucuronosyltransferase 1A1; C6G, codeine-6-glucuronide; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; M3S, morphine-3-sulfate; M6G, morphine-6-sulfate (Thorn et al., 2009).
The predominant metabolic pathway of oxycodone is through CYP3A4/5-mediated N-demethylation to noroxycodone (Figure 1.3) (Lalovic et al., 2004; Poyhia et al., 1991; Poyhia et al., 1993). CYP2D6 is responsible for the O-demethylation of oxycodone to form oxymorphone. Oxymorphone is a potent analgesic, with 64- and 3-times higher affinity for the MOR than oxycodone and morphine, respectively (Volpe et al., 2011). In addition, oxymorphone is glucuronidated by UGT2B7 to form the hydrophilic metabolite, oxymorphone-3-glucuronide that can be readily excreted from the body. Both oxymorphone and noroxycodone undergo further oxidation via CYP3A4/5 and CYP2D6, respectively to form noroxymorphone (Lalovic et al., 2006). The MOR affinity of noroxymorphone is 3- and 10-times higher than oxycodone and noroxycodone, respectively (Lalovic et al., 2006). Oxycodone and its metabolites are excreted in the urine and feces. Approximately 8.9% free and conjugated oxycodone, 23% noroxycodone, less than 1% oxymorphone, 14% noroxymorphone and 18% oxymorphone-3-glucuronide have been measured in the urine (Purdue Pharma Std., 2009). Both liver and renal impairments can have profound effects on the metabolism and excretion of oxycodone. The plasma oxycodone and noroxycodone concentrations are increased by approximately 50% and 20%, respectively in patients with renal impairment while the plasma oxymorphone concentration is decreased by 30% (Purdue Pharma Std., 2009). The accumulation of oxycodone is associated with an increased risk for toxicity (Gronlund et al., 2010; Gronlund et al., 2011; Kummer et al., 2011).
Figure 1.3 Metabolism of oxycodone in the liver.
Abbreviations: CYP3A4/5, cytochrome P450 3A4/5; CYP2D6, cytochrome P450 2D6, UGT2B7, UDP-glucuronosyltransferase 2B7 (Soderberg Lofdal et al., 2013).

1.1.5 Pharmacodynamics of Opioids

Opioids exert their analgesic effect in the peripheral and central nervous system. They activate the descending pathways that originate from the PAG. In the periphery, opioids depress both the presynaptic and postsynaptic membrane potential, which shortens the duration of the action potential that carries the pain signal to the dorsal horn (Macdonald and Werz, 1986). At the spinal cord, opioids decrease the excitability of the dorsal horn neurons. This inhibits the release of nociceptive neurotransmitters (substance P, acetylcholine, noradrenaline) that transmit the nociceptive signal to the brain (Sabbe and Yaksh, 1990; Yaksh, 1981). Opioids can activate the descending pathways that originate from the PAG, and thereby increase the release of nociceptive inhibitory chemical mediators onto the dorsal horn (Young-McCaughan and Miaskowski, 2001).

The respiratory center in the medulla receives peripheral input from chemoreceptors to control breathing. Both μ and δ receptors are located in the respiratory center (Peckys and Landwehrmeyer, 1999). By inhibiting neuronal excitability, opioids decrease the sensitivity of
chemoreceptors to changes in oxygen and carbon dioxide outside the normal concentration ranges (Bianchi et al., 1995; Santiago et al., 1977).

Sedation and emesis are two of the most common adverse effects following opioid use. Opioids may inhibit the mesencephalic reticular activating system in the medulla that is responsible for regulating arousal and sleep-wake transition (Young-McCaughan and Miaskowski, 2001). The μ, δ, κ receptors in the chemoreceptor trigger zone in the medulla are activated following opioid administration. Upon activation, the chemoreceptor trigger zone communicates with the area postrema to induce nausea and vomiting.

Opioids produce a number of other pharmacodynamic effects including miosis (pupillary constriction) via excitation of the parasympathetic nerves that innervate the pupil (Weinhold and Bigelow, 1993). In the gastrointestinal tract, opioid inhibit the release of acetylcholine in the myenteric plexus, which reduces biliary, pancreatic and intestinal secretions (Fox-Threlkeld et al., 1994; Mancini and Bruera, 1998). This slows intestinal movement leading to constipation.

1.2 Effect of Pharmacogenetics on the Efficacy and Safety of Opioids

1.2.1 Polymorphisms in Drug Metabolizing Enzymes

1.2.1.1 CYP2D6 Polymorphisms

Interindividual variability in opioid response can be due to differences in concentrations of either the parent opioid drug or its active metabolites at the site of action. The highly polymorphic CYP2D6 enzyme is of greatest interest because it metabolizes opioid analgesics such as codeine, oxycodone, hydrocodone and tramadol into the active analgesic forms morphine, oxymorphone, hydromorphone and O-desmethyltramadol (M1), respectively. Over 100 genetic variants in CYP2D6 have been identified with varying functional activity (Human Cytochrome P450 Allele Nomenclature Committee, 2014). The combinations of non-functional, reduced function and functional alleles result in a wide range of activity that can be segregated into four phenotypes: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultra-rapid metabolizer (UM). The CYP2D6 PM has two non-functional alleles within the CYP2D6 gene or arising from a complete gene deletion. The CYP2D6 IM has
two reduced function alleles or a combination of a nonfunctional with a functional or reduced function allele. The CYP2D6 EM has two functional alleles and CYP2D6 UM has functional duplications of CYP2D6 resulting in two or more functional alleles. The two phenotypes, CYP2D6 PM and UM, are of clinical significance because they are at the highest risk of opioid treatment failure or exaggerated opioid adverse events, respectively if the opioid needs to be bioactivated. The global distribution of CYP2D6 genetic variations is disproportionally distributed (Sistonen et al., 2007). The CYP2D6 PM phenotype occurs in 7-10% of Caucasians (Kosarac et al., 2009). The frequency of CYP2D6 UM in Western Europe is 5.45% (range 1-10% depending on the country of origin) (Ingelman-Sundberg, 2005), while its prevalence is higher in individuals with northeast African and Middle Eastern ancestry (ie. 16% in Ethiopians and 10% in Saudi Arabians) (Aklillu et al., 1996; McLellan et al., 1997).

A difference in opioid analgesic requirement may be indicative of variability in pain sensitivity. The CYP2D6 mRNA and protein have been detected in several regions of the brain including the hippocampus, thalamus, hypothalamus and the cortex (Siegle et al., 2001). CYP2D6 mediates the biotransformation of tyramine into endogenous morphine and dopamine (Funae et al., 2003; Hiroi et al., 1998; Yu et al., 2003). Excised ganglia from the mouse incubated with tyramine showed a concentration and time-dependent increase in endogenous morphine and dopamine production (Zhu et al., 2005). Morphine and dopamine production were diminished when incubated with quinidine, a CYP2D6 inhibitor (Zhu et al., 2005). Endogenous morphine colocalized with the MOR in areas of the brainstem involved in pain modulation (Stefano et al., 2000). Depletion of endogenous morphine in the brain increased nociceptive transmission (shorter latency) (Guarna et al., 2002). Collectively, endogenous morphine production mediated by CYP2D6 in animals plays a role in pain modulation. Thus CYP2D6 polymorphisms may impact pain sensitivity mainly because CYP2D6 is involved in endogeneous morphine formation in the brain.

It has been hypothesized that CYP2D6 PMs experience acute pain more often compared to EMs and UM because they may have a defect in the synthesis of endogenous morphine (Sindrup et al., 1993; Zahari and Ismail, 2014). Sindrup and colleagues evaluated the sensitivity to pain stimuli in 94 CYP2D6 EMs compared to 82 CYP2D6 PMs using the heat and pressure test
and the cold pressor test (Sindrup et al., 1993). Although the pain detection and tolerance thresholds did not differ between CYP2D6 EMs and PMs in the heat and pressure test, the peak pain rating and area under the pain rating-time curve from the cold pressor test in the PMs were significantly higher than that in the EM. A greater proportion of CYP2D6 PMs prematurely withdrew their hand during the cold pressor test due to intolerable pain compared to EMs.

A study in 236 adults undergoing surgery found that the CYP2D6 phenotype was a strong predictor of acute severe postoperative pain (Yang et al., 2012). CYP2D6 PMs were significantly more likely to suffer from severe postoperative pain compared to those who were IMs, EMs, or UM. On the other hand, CYP2D6 UMs may require less exogenous opioid for pain control due to the higher efficiency in endogeneous morphine synthesis (Candiotti et al., 2009). A study with 142 patients requiring morphine after undergoing elective surgery were divided into two groups, based on their morphine consumption, low morphine consumer and high morphine consumer (Candiotti et al., 2009). Low morphine consumers were more likely to be CYP2D6 UMs compared to high morphine consumers. CYP2D6 UMs required less morphine for the management of acute pain during the postoperative period compared to other CYP2D6 phenotypes.

The effect of cerebral CYP2D6 activity on the synthesis of morphine following peripheral codeine administration has also been recently demonstrated. Rats who had their cerebral CYP2D6 activity inhibited via intracerebroventricular injection of the CYP2D6 inhibitor propranolol experienced lower analgesia in the tail-flick test, brain morphine concentrations and brain morphine-to-codeine ratio compared to the controls (Zhou et al., 2013). Thus CYP2D6 polymorphisms likely determine the therapeutic efficacy and side effects of codeine.

The substantial variability in codeine, oxycodone, hydrocodone and tramadol pharmacokinetics due to differences in the ability to form the active metabolites has been extensively studied. In addition, CYP2D6 polymorphisms have a significant impact on opioid efficacy and susceptibility to toxicity (Wang et al., 2011). A study in 12 patients showed a 120-fold difference in the area under the concentration versus time curve (AUC) following codeine administration between CYP2D6 PMs and UM. Sindrup and colleagues noticed that all 12
CYP2D6 PMs were unable to produce morphine following codeine administration compared to 2 out of 12 CYP2D6 EMs (Sindrup et al., 1990). Since CYP2D6 PMs are devoid of functional CYP2D6 activity, they are at the highest risk for opioid analgesic treatment failure. Specifically, CYP2D6 PM is associated with poor analgesia in response to codeine (Chen et al., 1991; Poulsen et al., 1996; Sindrup et al., 1990; Sindrup et al., 1992), oxycodone (Kummer et al., 2011; Samer et al., 2010a; Stamer et al., 2013), and tramadol (Enggaard et al., 2006; Laugesen et al., 2005; Stamer et al., 2007) compared to those who were a CYP2D6 EM. Since the analgesic activity in CYP2D6 PMs may be reduced, administering an opioid that does not depend on the conversion by CYP2D6 to elicit its analgesic effect is recommended (MacDonald and MacLeod, 2010; Samer et al., 2010a; Tremlett et al., 2010).

On the other hand, individuals who are CYP2D6 UM s may produce higher amounts of the active metabolite and may experience exaggerated toxicity and death (Gasche et al., 2004; Koren et al., 2006). Increased plasma morphine concentrations, ranging from 50% up to 85% following codeine administration, have been reported in CYP2D6 UM s compared to EMs (Gasche et al., 2004; Kirchheiner et al., 2007). A higher proportion of CYP2D6 UM s experiencing CNS depression compared to EM has been reported (Kirchheiner et al., 2007). The increased prevalence of adverse effects in the CYP2D6 UM may be attributed to the significantly higher plasma morphine AUCs (Kirchheiner et al., 2007). Several case reports have demonstrated the clinical impact of codeine in CYP2D6 UM patients (Ciszkowski et al., 2009; Dalen et al., 1997; Gasche et al., 2004; Koren et al., 2006; Voronov et al., 2007). A CYP2D6 UM woman received 60 mg of codeine following a tooth extraction procedure. After 30 minutes, she experienced euphoria, dizziness, and visual disturbances (Dalen et al., 1997). The same symptoms were observed when she was readministered codeine. Gasche and colleagues described a CYP2D6 UM male with renal failure taking 25 mg of codeine three times a day for cough suppression (Gasche et al., 2004). He was concomitantly prescribed two CYP3A4 inhibitors, clarithromycin and voriconazole for the treatment of bilateral pneumonia. The patient became unconscious. Naloxone was immediately given and he regained consciousness. Toxicity may be attributed to a combination of CYP2D6 UM genotype, inhibition of CYP3A4 activity and reduced renal function. Recently, several case reports of codeine fatalities following tonsillectomy in young
children with obstructive sleep apnea syndrome have been described (Ciszkowski et al., 2009; Kelly et al., 2012). These case reports highlight the fact that codeine use in this population may not be safe, especially in CYP2D6 UM infants. The Clinical Pharmacogenetics Implementation Consortium (CPIC) published guidelines for pharmacogenetic testing and codeine dosing in an attempt to make the prescribing and use of codeine safe for patients (Crews et al., 2014).

The impact of CYP2D6 polymorphisms on the analgesic effects and toxicity of oxycodone has only been investigated in the last decade and conflicting evidence exists. A randomized crossover double-blind placebo-controlled study in 10 healthy volunteers administered oxycodone alone or after inhibition of CYP2D6 (with quinidine) and/or CYP3A (with ketoconazole) was conducted. In this study, CYP2D6 activity correlated with plasma oxymorphone concentrations and oxycodone experimental pain assessments (Samer et al., 2010a; Samer et al., 2010b). The AUC of oxymorphone was 83% lower in CYP2D6 PMs and 73% lower in EMs compared to UMs (Samer et al., 2010b). CYP2D6 UMs experienced increased analgesic efficacy as well as toxicity compared to PMs and EMs (Samer et al., 2010a). Oxycodone-induced toxicity was only observed in CYP2D6 UMs and/or after CYP3A4 inhibition (Samer et al., 2010a). In contrast, studies comparing carriers of CYP2D6 UM and EM genotype or concomitant administration of the CYP2D6 inhibitor quinidine failed to find any differences in pharmacological and adverse effects between the two groups, even though oxymorphone concentrations were significantly altered (Cleary et al., 1994; Gronlund et al., 2010; Gronlund et al., 2011).

Kirchheiner and colleagues evaluated the effects of CYP2D6 gene duplication on the pharmacokinetics and pharmacodynamics (assessed by cold pressure test, pupillometry and standardized adverse event recording) after a single dose of tramadol was given to 11 CYP2D6 UMs and 11 CYP2D6 EMs (Kirchheiner et al., 2008). CYP2D6 UMs had higher plasma levels of O-desmethyltramadol (M1) and better pain management compared to the CYP2D6 EMs (Kirchheiner et al., 2008). Half of the CYP2D6 UMs reported experiencing nausea compared to only 9% in the EM group suggesting that UMs were more sensitive to tramadol than EMs. Collectively, these studies clearly demonstrate that CYP2D6 UMs may be at a higher risk for opioid adverse events. The prevalence of adverse events may occur more frequently in ethnic
groups that have a higher proportion of CYP2D6 UM's such as in North African and Middle Eastern populations (Sistonen et al., 2007).

The importance of the interplay between CYP2D6 polymorphisms and drug interactions on the pharmacokinetics, pharmacodynamics and susceptibility to opioid toxicity has received much attention in recent years. When the compensatory CYP3A4 pathway is inhibited by a concomitant medication, there may be an accumulation of the parent opioid (Madadi et al., 2010; Samer et al., 2010a). A fatal report of a fatality in a child who was prescribed hydrocodone for a respiratory tract infection clearly demonstrated the importance of considering both genetic and non-genetic factors when assessing the influence of CYP2D6 polymorphisms on opioid analgesic response and adverse events (Madadi et al., 2010). This child was genotyped to be a CYP2D6 PM, suggesting that she had reduced CYP2D6 activity to metabolize hydrocodone to hydromorphone. Consistent with the genetic analysis, hydromorphone was undetected in the plasma. Hydrocodone is also metabolized by CYP3A4 into norhydrocodone. However, this child was also prescribed clarithromycin, a CYP3A inhibitor, which may have led to increased and toxic levels of hydrocodone (Madadi et al., 2010). Both CYP2D6 and CYP3A are involved in the metabolism of many opioids such as codeine, oxycodone, tramadol and hydrocodone. In a pharmacokinetic study, Samer and colleagues showed that these two enzymes are inextricably linked (Samer et al., 2010b). When the activity of one enzyme is inhibited, the metabolic rate of the other enzyme increases. Other reports of a concomitant use of a CYP2D6 inhibitor leading to opioid-induced toxicity have also been reported (Samer et al., 2010a; Sindrup et al., 1992). Concurrent administration of CYP2D6 inhibitors can imitate genetically-mediated variation in opioid metabolism. For example, concomitant use of CYP2D6 inhibitors such as paroxetine, fluoxetine, and citalopram will convert an individual to become a CYP2D6 PM regardless of their CYP2D6 genotype (Jin et al., 2005; Lohmann et al., 2001). In this case, the CYP3A pathway is important in the metabolism of the parent opioid to prevent it from building up in the body.

1.2.1.2 UGT2B7 Polymorphisms

The UGT2B7 enzyme is responsible for mediating the glucuronidation of codeine, morphine, and oxymorphone into C6G, M3G and M6G, and oxymorphone-glucuronide,
respectively (Coffman et al., 1997; Coffman et al., 1998; Kirkwood et al., 1998). Polymorphisms in the UGT2B7 gene may lead to interindividual variability in opioid and opioid glucuronide pharmacokinetics. Variability in drug response can also be altered given that glucuronidated metabolites are typically inactive compared to the parent opioid. However, the morphine metabolite M6G is more potent than morphine (Paul et al., 1989). The contribution of UGT2B7 polymorphisms to the interindividual differences in opioid response has not been thoroughly investigated.

Polymorphisms in UGT2B7 may have important clinical consequences, especially in the morphine pathway. Two variants of UGT2B7 have been identified, which differ by either having a histidine or tyrosine at amino acid residue 268. These variants, referred to as UGT2B7*1 and UGT2B7*2 respectively, arise from a C to T transversion at nucleotide 802 (Bhasker et al., 2000). Ethnic variations in UGT2B7 polymorphisms have been reported. There is approximately equal distribution of UGT2B7*1 and UGT2B7*2 allele in the Caucasian population (Bhasker et al., 2000). UGT2B7*1 has been reported to be 10-fold more prevalent in the Japanese population (Bhasker et al., 2000), while the UGT2B7*2 is rare in the Chinese and West African population (Mehlotra et al., 2007; Deng et al., 2013).

Despite the role of UGT2B7 in mediating the formation of M3G and M6G, conflicting findings have been published by several groups when investigating the functional effect of the UGT2B7*2 variant on morphine glucuronidation (Bhasker et al., 2000; Court et al., 2003). Both in vitro and in vivo studies showed that the UGT2B7 activity was not altered by the His268Tyr (UGT2B7*2) substitution (Bhasker et al., 2000; Coffman et al., 1998; Ross et al., 2005). Liver microsomes from Caucasian donors were prepared to measure the rate of microsomal aldosterone, menthol and morphine glucuronidation (Bhasker et al., 2000). Liver samples were also genotyped for the UGT2B7*1 and UGT2B7*2 variants. The rate of glucuronidation of all three substrates was not significantly different between the two variants. Similarly, a study with the UGT2B7*1 and UGT2B7*2 cDNA cloned and stably expressed in HK 293 cells failed to show a difference in the glucuronidation of morphine (Coffman et al., 1998).
To add to these complexities, serum morphine and morphine glucuronide metabolite concentrations have not been shown to correlate with pain relief or side effects in the clinical setting (Klepstad et al., 2003; Quigley et al., 2003). A prospective study was conducted in cancer patients prescribed morphine to correlate UGT2B7 genotype and morphine and morphine glucuronide concentrations (Ross et al., 2005). The patients were divided into two groups: patients who received analgesic benefits with morphine and those who did not tolerate morphine and had to be switched to an alternative opioid. There was no correlation between UGT2B7 genotype and morphine or morphine glucuronide levels. In addition, no significant differences in UGT2B7 genotype or allelic frequencies were observed between those who had or did not have their morphine switched. A study consisting of 175 Norwegian cancer patients receiving chronic oral morphine therapy measured the serum morphine and morphine glucuronide concentration 1 to 2 hours after morphine administration. There was no correlation between UGT2B7 genotype and morphine glucuronide-to-morphine serum ratios, suggesting that UGT2B7 polymorphisms may not be an important contributor to the variability in serum morphine and morphine glucuronide concentrations (Holthe et al., 2003).

One study reported an increase in the M6G-to-morphine ratio in individuals who were homozygous for the UGT2B7*2 allele (Sawyer et al., 2003). There was a significant trend for decreased M6G-to-morphine ratios in *2/*2, *1/*2 and *1/*1 patients. A similar trend was observed when assessing the M3G-to-morphine ratio. However, the results from this study could not be replicated in studies of similar size (De Gregori et al., 2013). Therefore, the influence of UGT2B7 polymorphisms on opioid response remains to be elucidated.

1.2.2 OPRM1 Polymorphisms

The experience, sensation and response to pain can be influenced by genetic polymorphisms in a number of pain targets. Out of all the pain targets identified, the association between polymorphisms in OPRM1, encoding for the MOR and opioid response and safety is most extensively studied, with no definitive conclusions. The MOR is the primary site of action for opioid analgesics. Approximately 100 variants have been identified (Lotsch and Geisslinger, 2005). Many of the mutations are in the promoter, coding regions and introns of the OPRM1 gene (Lotsch and Geisslinger, 2005). The most commonly identified single
nucleotide polymorphism (SNP) is **OPRM1 A118G** (A to G substitution in nucleotide 118 of exon 1), which results in an amino acid substitution from asparagine to aspartate at position 40 (N40D). This amino acid change leads to a loss of a putative N-glycosylation site at the N-terminal of the MOR (Lotsch and Geisslinger, 2005). The allelic frequency of the **OPRM1 A118G** polymorphism varies between different ethnic groups, ranging from 10% to 32% (LaForge et al., 2000).

The **OPRM1 A118G** SNP significantly alters mRNA and receptor expression but changes in receptor signaling are inconsistent (Befort et al., 2001; Beyer et al., 2004; Bond et al., 1998; Zhang et al., 2005). OPRM1 mRNA expression in postmortem human brain tissues revealed that individuals who were homozygous for the A allele (**OPRM1 118AA**) was 1.5-fold higher than those who were homozygous for the G allele (**OPRM1 118GG**) (Zhang et al., 2005). Chinese hamster ovary cells expressing this SNP had 1.5-fold and 10-fold lower OPRM1 mRNA and protein levels, respectively, compared to control cells (Zhang et al., 2005). Although the binding affinities of many endogenous opioids (enkephalin and dynorphin) and exogenous opioids (morphine, fentanyl, methadone and naloxone) were not affected in AV-12 cells transfected with the mutant receptor, β-endorphin affinity was 3.5- times higher compared to control cells (Bond et al., 1998). However, another study observed a similar binding affinity for β-endorphin between the mutant and wild-type receptor (Beyer et al., 2004). The signaling pathway downstream from the mutated MOR on HEK23 cells remained unaltered following stimulation by [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO) (Befort et al., 2001; Beyer et al., 2004). Furthermore, the production of cAMP following activation of the mutant receptor was not significantly different compared to wild-type receptor (Beyer et al., 2004).

It has been speculated that the **OPRM1 A118G** polymorphism alters the clinical response of opioids and is protective against opioid-induced toxicity (Lotsch et al., 2002; Lotsch and Geisslinger, 2005). To date, most studies in cancer and perioperative patients suggest that wild-type patients (**OPRM1 118AA**) require significantly less morphine (Chou et al., 2006b; Chou et al., 2006a; Hayashida et al., 2008; Klepstad et al., 2004; Sia et al., 2008) or M6G (Lotsch et al., 2002; Lotsch and Geisslinger, 2005; Skarke et al., 2003a) compared to heterozygous or homozygous variant carriers (**OPRM1 118AG/G**). A study in 99 cancer patients requiring chronic
morphine therapy found that patients with the *OPRM1 118GG* genotype (n=4) required more morphine to achieve pain control compared to those who were *OPRM1 118AG* (n=17) and *OPRM1 118AA* (n=78) (Klepstad et al., 2004). Even after controlling for non-genetic factors such as duration of morphine treatment, performance status, and time since diagnosis and adverse symptoms, the influence of *OPRM1* polymorphism on morphine efficacy in cancer patients remained significant. Sia and colleagues investigated whether the *OPRM1 A118G* polymorphism contributes to variability in response to morphine in 588 women after Caesarian section (Sia et al., 2008). Pain scores, the prevalence and severity of nausea and vomiting, and the total dose of morphine self-administered during the first 24 hours after Caesarian section were collected. Women with the *OPRM1 118AA* genotype consumed a lower amount of morphine compared to those who were AG or GG. In parallel, the *OPRM1 118AA* genotype had the lowest pain score while those who were *OPRM1 118GG* scored the highest. Furthermore, *OPRM1 118AA* women had the highest incidence of nausea compared to the other two genotypes, suggesting a protective role of the variant allele in morphine-induced toxicity.

There are limited studies available for opioids other than morphine. The analgesic effect of M6G was decreased in *OPRM1 118GG* homozygous patients compared to those who were homozygous wild-types (Lotsch et al., 2002). Following M6G administration, the prevalence of vomiting was less frequent in healthy volunteers who carried at least one copy of the G allele compared to the homozygous wild-types (Skarke et al., 2003a). Several recent studies have sought to elucidate the association between the *OPRM1 A118G* SNP and pain management following oxycodone administration in postoperative patients and healthy volunteers, with contradictory results (Klepstad et al., 2011; Zwisler et al., 2010; Zwisler et al., 2011). Two hundred sixty eight postoperative patients were asked to rate their pain level following oxycodone administration during a 24-hour postoperative observation period (Zwisler et al., 2011). The percentage of nonresponders, patients who felt that they had inadequate analgesic relief with oxycodone or were given morphine during the observatory period, was not significantly different between the homozygous wild-type group and carriers of the variant allele. In addition, pain measurement and adverse drug reactions were not significantly different between the two groups. In contrast, a study evaluating experimental pain in healthy
subjects (n=33) found that carrying at least one copy of the G allele was associated with reduced analgesic effect when measured using the electrical stimulation test but not in the cold pressor test (Zwisler et al., 2010). Carriers of the G variant had a reduced ability to keep focus compared to the homozygous wild-type. However, the total sum of adverse drug reactions associated with oxycodone use was not statistically different between the two groups. The contradictory findings can be explained by differences between experimental pain and clinical postoperative pain. Healthy volunteers who are subjected to experimental pain are tightly monitored and psychological factors associated with pain sensation are usually eliminated. However, postoperative patients may be experiencing pain for a longer period of time in an uncontrolled environment and may be prescribed many concomitant medications. The largest multicenter study conducted to date investigated the influence of 112 SNPs in 25 candidate genes proposed to influence opioid efficacy in 2201 cancer patients (Klepstad et al., 2011). The cancer patients were taking morphine (36%), fentanyl (30%), oxycodone (19%) or other opioids (10%) as their primary opioid for cancer-related pain. The OPRM1 A118G polymorphism failed to predict opioid dose, refuting the need to use opioid pharmacogenetics to guide pain therapy.

A recent meta-analysis evaluating the influence of the OPRM1 A118G polymorphism in predicting both opioid analgesia and side effects found no association between OPRM1 A118G polymorphism and opioid dose requirement (Walter and Lotsch, 2009). Only a few side effects such as nausea, vomiting, constipation and sedation have been associated with OPRM1 A118G. This reflects the lack of clear evidence to support the use of the OPRM1 A118G SNP to guide pain therapy (Walter and Lotsch, 2009). Also Walter and colleagues addressed the pitfalls of genetic research, which included population heterogeneity, inappropriate choice of genetic variants, small sample size, complexity of the pain phenotype, presence of concomitant medications, different intensities of symptoms other than pain, improper statistical analysis and publication bias. Typically, most studies only examine the influence of one or a few SNPs within a gene on opioid efficacy. The reality is that two or more genes may have joint effects and variations in these genes together may better predict opioid response or safety.

A study in 145 patients receiving morphine for cancer-related pain found a decreased morphine requirement in individuals who were OPRM1 118AA and ABCB1 3435TT, illustrated
by a gene dose effect \((118\text{AA}/3435\text{TT} < 118\text{AG}/3435\text{CT} < 118\text{GG}/3435\text{CC})\) (Campa et al., 2008). Similarly, other studies have explored the joint effect of \(\text{OPRM1 A118G}\) and catechol-\(\text{O-}
\)methyltransferase (\(\text{COMT}\)) Val158Met on the morphine dose requirement for pain management in cancer patients (Reyes-Gibby et al., 2007). The \(\text{COMT}\) enzyme, encoded by the \(\text{COMT}\) gene is involved in the metabolism of catecholamines including dopamine, epinephrine and norepinephrine (Diatchenko et al., 2007; Zubieta et al., 2003). These catecholamines have been shown to be influence pain perception (Diatchenko et al., 2007; Rakvag et al., 2005; Rakvag et al., 2008). The Val158Met polymorphism reduces \(\text{COMT}\)'s activity leading to decreased capacity of the mu-opioid neurotransmitter system to respond to a painful stimulus (Zubieta et al., 2003). Individuals with the \(\text{COMT} \text{Val/Val}\) and \(\text{Val/Met}\) genotype required 63% and 23% more total morphine within a 24 hour period compared to those with the \(\text{Met/Met}\) genotype, respectively (Reyes-Gibby et al., 2007). A gene dose effect was also seen in individuals who were \(\text{OPRM1 118GG}\) requiring 93% more total morphine within a 24 hour period compared to those with the \(\text{AA}\) genotype \((\text{GG} > \text{AG} > \text{AA})\). Individuals who had the combination genotype \(\text{COMT 158Met/Met}\) and \(\text{OPRM1 118AA}\) required the least amount of morphine compared to all other genotype combinations. Even after controlling for non-genetic factors such as duration of morphine treatment, time since cancer diagnosis, age, serum albumin and serum creatinine, the joint effect of \(\text{COMT 158Met/Met}\) and \(\text{OPRM1 118AA}\) remained a significant predictor of morphine dose requirement. This study illustrates the importance of using large human clinical studies to explore the joints effect of genetic variations in the pain pathway, drug-metabolizing enzymes and transporters in addition to non-genetic factors in order to understand how each of them may influence the clinical efficacy of opioids.

1.2.3 \(\text{ABCB1}\) Polymorphisms

The transport of drugs by \(\text{P-glycoprotein (P-gp)}\) out of tissues can influence the rate and extent of drug absorption, distribution and elimination. Its activity in the small intestine, liver and kidneys is an important determinant of the absorption and clearance of the parent opioid and its metabolite from the body (Shitara et al., 2006). Its expression at the BBB can impact the response and susceptibility to opioid toxicity (Lin and Yamazaki, 2003). The importance of \(\text{P-gp}\)
in determining opioid exposure in the CNS is discussed in further detail in Section 1.5. Consequently, genetic variability in the \textit{ABCB1} gene encoding P-gp may influence opioid exposure in the CNS.

The two most extensively studied mutations are the \textit{ABCB1 G2677T/A} and \textit{C3435T} (Leiri et al., 2004). The transversion of G to T or A at nucleotide 2677 in exon 21 results in an amino acid change from alanine to serine or threonine, respectively at position 893. The transversion of C to T at nucleotide 3435 in exon 26 does not change the amino acid sequence. Both \textit{G2677T/A} and \textit{C3435T} are in linkage disequilibrium such an individual who is homozygous variant at \textit{G2677T/A} will also most likely be homozygous variant at \textit{C3435T} (Kim et al., 2001; Siegmund et al., 2002; Tanabe et al., 2001). The allelic frequency distribution of \textit{ABCB1 G2677T/A and C3435T} varies between different ethnic groups. The frequency of the \textit{ABCB1 3435 C} allele has been reported to be 50 to 60% in Caucasians (Ameyaw et al., 2001), 34 to 63% in Asians (Sakaeda et al., 2001; Tang et al., 2002) and 73 to 90% in Africans (Ameyaw et al., 2001; Schaeffeler et al., 2001). Africans are more likely to have the CC and CT genotype compared to Caucasians and Asians (Ameyaw et al., 2001; Schaeffeler et al., 2001). The frequency of \textit{ABCB1 2677 G} allele has been reported to be 57% in Caucasians and 43% in Japanese (Ameyaw et al., 2001; Cascorbi et al., 2001).

The effect of these SNPs, mainly \textit{C3435T}, on P-gp mRNA and protein expression and activity has been examined in detail (Calado et al., 2002; Hoffmeyer et al., 2000; Lown et al., 1997; Meissner et al., 2002; Siegmund et al., 2002; Tanabe et al., 2001). Studies have shown a significant trend for decreased P-gp mRNA and protein levels in small intestine (Hoffmeyer et al., 2000; Lown et al., 1997; Siegmund et al., 2002), heart (Meissner et al., 2002), kidney (Siegmund et al., 2002), placenta (Hitzl et al., 2004) and lymphocytes (Calado et al., 2002) in \textit{3435 CC, CT and TT} individuals. Using immunohistochemistry and Western blotting, the P-gp protein levels were quantified in duodenal biopsies (Hoffmeyer et al., 2000). The homozygous T allele was associated with more than 2-fold lower P-gp expression compared to the homozygous C allele (CC > CT > TT). An inverse relationship between duodenal P-gp expression and digoxin plasma concentrations was observed, with persons with homozygous T alleles having higher digoxin plasma concentrations compared to those who were homozygous for the
C allele. This implies that genetic variability in \textit{ABCB1} may have the ability to influence the bioavailability of orally administered drugs by restricting or facilitating intestinal absorption. Similar to its effect on P-gp protein levels, P-gp mRNA was the lowest in individuals who homozygous for the T allele and the highest in homozygous wild-types (Drach et al., 1996; Hitzl et al., 2001). The lower mRNA levels may be a result of decreased mRNA stability in the T allele carriers (Wang et al., 2005). Several knowledge gaps still need to be addressed: 1) How does the \textit{ABCB1} C3435T polymorphism influence the expression of P-gp at the BBB? 2) What effect does the \textit{ABCB1} G2677T/A polymorphism have on P-gp mRNA and protein levels? 3) Is the \textit{in vitro} and \textit{in vivo} transport of opioid affected by these \textit{ABCB1} polymorphisms?

Since many of the SNPs in the \textit{ABCB1} gene are in strong linkage disequilibrium, studies examining the effect of \textit{ABCB1} haplotypes are more likely to accurately predict P-gp expression and function compared to assessing individual SNPs. Although the absorption of loperamide was not significantly associated with the \textit{ABCB1} C3435T variant alone, when haplotypes were assessed, the loperamide plasma concentration in subjects carrying the \textit{ABCB1} haplotype 2677G/3435T was approximately 1.5-fold higher than in non-carriers of this haplotype (Skarke et al., 2003b). The \textit{ABCB1} haplotype 2677G/3435C was associated with a lower need for antiemetic treatment with ondansetron following morphine administration compared to the other haplotypes (Coulbault et al., 2006). A pharmacokinetic modeling study suggested that individuals with the \textit{ABCB1} 3435TT genotype may have up to 7 fold higher morphine concentration in the cerebrospinal fluid (CSF) compared to those homozygous for the wild-type (Meineke et al., 2002). Thus, a lower requirement for ondansetron among patients with the \textit{ABCB1} haplotype 2677G/3435C may suggest that the penetration of morphine into the brain is significantly lower compared to non-carriers of this haplotype. However, neither \textit{ABCB1} G2677T/A nor C3435T polymorphisms were not associated with morphine requirement following abdominal surgery (Coulbault et al., 2006).

The \textit{ABCB1} G2677T/A and C3435T SNPs are also in strong linkage disequilibrium with other SNPs such as the C1236T SNP (Kim, 2002; Kroetz et al., 2003). Examining the effect of these haplotypes will likely be more informative. Various studies have reached different conclusions regarding the influence of these haplotypes on methadone dose requirement.
(Coller et al., 2006; Lotsch et al., 2006). Methadone dose requirement was investigated in 60 methadone maintenance patients. The \textit{ABCB1} haplotype in five SNPs (61/1199/1236/2677/3435) revealed that patients carrying two copies of the wild-type haplotype \((A16/G1199/C1236/G2677/C3435)\) required a significantly higher methadone dose compared to carriers of one copy or none of the wild-type haplotype (Coller et al., 2006). This suggests that homozygous wild-type haplotype patients might have higher P-gp expression and function and thereby reduced the central levels of methadone compared to the other combinations. When methadone doses were compared between groups for the haplotype variant at only position 2677 and 3435, patients who were carriers for the \textit{A16/G1199/C1236/T2677/T3435} required significantly less methadone during treatment compared to non-carriers. Whether \textit{ABCB1} polymorphisms and haplotypes predict opioid requirements and adverse events remains to be elucidated in future clinical studies.

1.3 Patterns of Opioid Use

1.3.1 Worldwide Opioid Consumption

The demand for opioid production has remained high for the last 20 years (International Narcotics Control Board, 2012). Since the demand for morphine production is the highest out of all of the opioids, it is not surprising that it is also the most widely used and prescribed narcotic drug in medical practice. Australia, France, Spain and Turkey are the top four producers of morphine, accounting for about 95% of the global morphine production (International Narcotics Control Board, 2012). In 2010, it was estimated that 416 tons of morphine were produced worldwide (International Narcotics Control Board, 2012). The global consumption of morphine for the treatment of severe pain increased by 6-fold over the last 20 years. The consumption of morphine is distributed unevenly worldwide. Australia, Canada, Japan, New Zealand and the United States account for more than 92% of the morphine consumption (International Narcotics Control Board, 2012).

Approximately 94% of the morphine produced worldwide is manufactured into codeine (International Narcotics Control Board, 2012). Codeine is the second most widely prescribed narcotic drug in the pediatric population after morphine (de Lima et al., 1996). Since majority of
drugs used for pain management has not been approved in the pediatric population, the common perception that codeine is a weak opioid with a low risk of adverse effects has advocated its off-label use in this population (Conroy and Peden, 2001).

The global manufacture and consumption of the semi-synthetic opioids, oxycodone, hydrocodone and hydromorphone are on the rise. They are commonly prescribed for the management of moderate to severe pain (International Narcotics Control Board, 2012). Canada is the second major consumer of oxycodone after the United States (International Narcotics Control Board, 2012).

1.3.2 Opioid Use During Pregnancy

The use of drugs during pregnancy has been a concern for both health care providers and pregnant mothers, given the fear of harming the unborn child. Despite this, many pregnant women require the use of drugs to manage their conditions during pregnancy. Opioids are the most commonly used medication during pregnancy. In a Canadian survey, 42% of pregnant women reported using opioid at least once in their pregnancy (Garriguet, 2006). The most common opioid dispensed during pregnancy was hydrocodone (6.7%), followed by codeine (6.1%) and oxycodone (2%) (Garriguet, 2006). These opioids (hydrocodone, codeine and oxycodone) were used for less than one week. On the other hand, morphine, fentanyl, methadone, and oxymorphone were used for a longer period of time (Garriguet, 2006). Although there are a limited number of studies investigating the pregnancy outcome following opioid use, none of them reported an association between short term opioid use and major malformation (Babb et al., 2010; Broussard et al., 2011; Juurlink et al., 2012; Nezvalova-Henriksen et al., 2011). However, with chronic use of opioids during late pregnancy, there is a risk for neonatal abstinence syndrome (NAS) in the first days of life (Liu et al., 2010; Iseman et al., 2011). NAS is characterized by poor feeding, irritability, sweating, and vomiting (Grim et al., 2013; Jones et al., 2014). Thus, the lowest effective dose of opioid should be used for the shortest amount of time in order to minimize the risk for NAS (Babb et al., 2010).
1.3.3 Opioid Use During Breastfeeding

Over the last decade the rate of breastfeeding has been steadily increasing (Center for Disease Control and Prevention (CDC), 2013; Health Canada, 2013). Health Canada recommends that in the absence of any contraindications, women should breastfeed their infants for at least the first 6 months of life (Health Canada, 2013). In addition, breastfeeding with complementary foods should be continued for up to two years of age (Health Canada, 2013). Human milk contains substances, nutrients, and immunological constituents that are essential for supporting the optimal growth of infants. There are many benefits associated with breastfeeding. The colostrum, the mother’s first milk, coats the newborn’s gut and decreases the gut’s permeability to pathogens (Wold and Adlerberth, 2000). Breast milk, containing an array of immunoglobulins, oligosaccharides, and cytokines, protects the newborn infant against gastroenteritis, upper and lower respiratory tract infection, acute otitis media, urinary tract infections, and necrotizing enterocolitis (Civardi et al., 2014; Dai and Walker, 1999; Lonnerdal, 2003; Peterson et al., 2013; Wold and Adlerberth, 2000). Despite the unquestionable benefits of breastfeeding, many mothers are concerned about taking drugs during breastfeeding. Approximately 30 to 40 percent of Canadian nursing women are prescribed opioid-containing medications during the postpartum period for the management of pain following Caesarian section or episiotomy (Health Canada, 2013). Therefore, it is important to balance pain relief with maternal and neonatal safety. Although it may be safe for the mother, the neonatal safety of maternal opioid use has not been thoroughly assessed.

1.3.4 Detection of Opioids in Postmortem Samples

Non-medical use of prescription opioids is a public health concern with tremendous economic, social and medical costs. With an increasing number of people using opioids for pain relief, there is an increased risk for opioid abuse, misuse and overdose (Atluri et al., 2014). Increased opioid consumption has also been associated with an increased number of fatalities related to opioids (Center for Disease Control and Prevention (CDC), 2012). It is crucial to identify factors that contribute to opioid-related fatalities in order to develop prevention and management strategies. The introduction of long-acting oxycodone to the Ontario drug formulary was identified as an important contributor to opioid-related mortality in Ontario,
Canada (Dhalla et al., 2009). Furthermore, oxycodone was the most common opioid involved in opioid-related fatalities in Ontario, followed by morphine and methadone (Madadi et al., 2013). Interestingly, oxycodone was associated with the highest proportion of both accidental and suicidal deaths (Madadi et al., 2013). Inappropriate route of opioid administration (i.e., injection, inhalation, or patch ingestion), opioid switching, and being involved in the correctional system have been associated with opioid-related fatalities (Madadi et al., 2013). Opioid-related fatalities often involve other drugs acting on the CNS such as alcohol, benzodiazepines, and tricyclic antidepressants (Cone et al., 2004). The reasons for polydrug abuse behavior are complex and warrant further investigation. Unfortunately, using multiple centrally acting drugs concurrently with opioids can lead to serious toxicity and even death.

1.4 Maternal and Neonatal Safety of Opioid Use During Lactation

1.4.1 Transfer of Opioids into Breast Milk and Associated Neonatal Outcomes

The risk for opioid-induced toxicity in the nursing infant may depend on the amount of opioid excreted into the human milk, the metabolic capacity of the infant, and the risk for toxicity at this exposure level. The transfer of opioids into breast milk is usually by passive diffusion. The rate of diffusion depends on several physiochemical properties such as molecular weight, degree of lipophilicity, extent of plasma protein binding, bioavailability, and degree of ionization (Ito, 2000). Generally, drugs that have a low molecular weight, are highly lipophilic, have moderate-to-high bioavailability, are moderately protein bound and uncharged are more readily excreted into the breast milk.

Opioids are small molecules that are highly lipophilic, and tend to have high oral bioavailabilities. They are moderately protein bound such that there may be a sufficient amount of unbound drug transferred into the breast milk. Since breast milk is slightly more acidic (pH 7.2) than the blood (pH 7.4), and opioids are weak bases, they become charged and trapped upon entry into the milk compartment. Due to the ion-trapping effect, the half-life of opioids in breast milk is prolonged, possibly increasing the risk for neonatal adverse effects. Since the metabolic capacity of the newborn’s kidneys and liver is reduced, there may be slow accumulation of opioids through breastfeeding (Bouwmeester et al., 2003).
1.4.2  Morphine Use During Breastfeeding

Low amounts of morphine were detected in the breast milk of mothers who received morphine for post-Caesarian section analgesia (Feilberg et al., 1989; Robieux et al., 1990; Wittels et al., 1990). Higher morphine milk concentrations have been detected in mothers given intravenous or oral morphine compared to those given epidural morphine (Wittels et al., 1990; Zakowski et al., 1993). Maternal use of morphine during breastfeeding has been associated with neonatal CNS depression and drowsiness. Newborn infants have heightened sensitivity to the central effects of morphine even when small doses of morphine are given (Bouwmeester et al., 2003; Koren et al., 1985; Way et al., 1965).

When a mother was using a low morphine dose during breastfeeding, her full term infant experienced unexplained apnea and bradycardia with cyanosis during the first week of life (Meny et al., 1993). Morphine was not detected in a breast milk sample taken 108 hours following the mother’s last dose. However, the neonatal plasma morphine concentration was 1.2mcg/L, which suggested that the plasma clearance was significantly reduced and possibly led to the accumulation of morphine in the newborn (Naumburg et al., 1987). One case report of a mother who was prescribed morphine 5mg q6h for 10 days reported no adverse effects in the nursing infant despite detecting clinically significant serum morphine levels in the infant (Robieux et al., 1990). In most cases, the levels of morphine in breast milk are expected to be relatively low and to have negligible effects on the nursing infant (Wittels et al., 1990).

1.4.3  Codeine Use During Breastfeeding

Codeine is commonly prescribed for the management of pain associated with childbirth. Despite limited published data, the American Academy of Pediatrics listed codeine as compatible with breastfeeding (American Academy of Pediatrics (AAP), 2001). These guidelines were based on three small studies that detected low or negligible amounts of codeine and/or morphine in the breast milk (Findlay et al., 1981; Meny et al., 1993; Sapeika, 1947).

In the first study, codeine or morphine was undetected in the breast milk four hours after a single dose of 65 mg was given to breastfeeding mothers (Sapeika, 1947). Findlay and colleagues detected higher codeine and morphine concentrations in the milk compared to that
measured in the plasma in the nursing mothers (Findlay et al., 1981). However, codeine was still listed as compatible with breastfeeding because breast milk codeine and morphine levels were too low to have an effect on the nursing infant (Findlay et al., 1981). Lastly, codeine and morphine concentrations were measured in the breast milk of and in the plasma of term neonates following maternal codeine use (Meny et al., 1993). Neonatal outcomes were also collected. None of the neonates exhibited adverse events and the neonatal plasma morphine concentrations were below the therapeutic range observed in infants given morphine (Lynn and Slattery, 1987; Meny et al., 1993).

In 2006, the first case of fatal respiratory depression in a newborn infant who was breastfed by a CYP2D6 UM mother taking a conventional dose of codeine was reported (Koren et al., 2006). Toxic morphine levels were detected in neonatal postmortem blood. The morphine concentration measured in breast milk was four-fold higher than any previous report. The mother was genotyped to be a CYP2D6 UM, which suggested that she was excreting higher than expected amounts of morphine into her breast milk that resulted in morphine intoxication in her newborn infant (Koren et al., 2006). After this case was published in 2006, regulatory agencies such as the United States Food and Drug Administration (FDA) and Health Canada issued public health advisories to warn mothers and physicians that codeine may not be safe for all neonates during breastfeeding, especially if the newborn infant is nursed by a CYP2D6 UM mother (U.S Food and Drug Administration (FDA), 2007; Health Canada, 2008).

A case-control study was performed on 72 mother-infant pairs exposed to codeine during breastfeeding (Madadi et al., 2009b). In this study, a dose-response relationship between maternal codeine dose and neonatal opioid toxicity was observed. In other words, mothers who reported neonatal CNS depression consumed on average a 59% higher daily dose of codeine per kg of maternal weight compared to those mothers who did not report neonatal CNS depression. In addition, there was concordance between maternal and neonatal CNS depression. Mothers of breastfed infants who experienced life-threatening CNS depression were more likely to be a CYP2D6 UM compared to mothers who were breastfeeding asymptomatic infants. As a result of gradual accumulation of morphine in breastfed infants, it
has been suggested that maternal codeine use should be limited to 3 or 4 days to avoid neonatal morphine poisoning (Madadi et al., 2007; Madadi et al., 2009b).

A physiologically-based pharmacokinetic modeling study was conducted to identify a combination of risk factors that may put the breastfed neonate at risk for morphine accumulation and morphine-induced toxicity (Willmann et al., 2009). Maternal CYP2D6 genotype, maternal codeine dose, duration of codeine use, and maternal and neonatal metabolic clearance capacity were identified as critical factors in predicting neonatal CNS depression (Willmann et al., 2009). Thus the interplay between clinical, developmental and genetic factors may protect the nursing infant from opioid-induced toxicity.

The current Motherisk guidelines for codeine use during breastfeeding recommend using a low dose of codeine for the shortest amount of time to prevent neonatal morphine toxicity (Madadi et al., 2007; Madadi et al., 2009a). It is very important for the mother and the prescriber to monitor for signs and symptoms of CNS depression in the nursing newborn (Madadi et al., 2009a). Although pretesting all mothers who plan to breastfeed before prescribing codeine may help to identify those mothers and infants who are at risk of experiencing opioid-induced toxicity, this intervention would be expensive and impractical (Madadi et al., 2012; MacDonald and MacLeod, 2010). Since the pharmacokinetics of morphine are much more predictable than codeine, the most logical solution may be to restrict the use of codeine during breastfeeding and give these mothers morphine instead (Kirchheiner et al., 2007; Lotsch et al., 2009). Until further research is available to understand the safety of codeine in adults, children and newborns, physicians need to warn mothers taking codeine about the risk for opioid-induced toxicity in their nursing child.

1.4.4 Oxycodone Use During Breastfeeding

In response to the death of a neonate breastfed by a CYP2D6 UM mother taking codeine (described above), some clinicians are replacing codeine with oxycodone for the management of moderate pain in the postpartum period. Clinical studies examining the outcomes of neonates breastfed by oxycodone-prescribed mothers are lacking. Like codeine, oxycodone is a CYP2D6 substrate (Lalovic et al., 2004; Lalovic et al., 2006). Although the analgesic effect of
oxycodone is less dependent on the activity of CYP2D6 compared to codeine, O-demethylation produces the more potent CYP2D6 metabolite, oxymorphine compared to morphine (Figure 1.3). Furthermore, the overall contribution of the CYP2D6 genotype in predicting efficacy and safety in breastfeeding mothers and their infant is unknown. Therefore, the replacement of codeine with oxycodone does not seem rational nor help minimize the risk for serious adverse events in the neonate.

Seaton and colleagues detected oxycodone in breast milk of all the mothers (n=50) in their study, suggesting that oxycodone was transferred into the milk regardless of the dose of oxycodone that was taken in a 24-hour period (Seaton et al., 2007). It was still detectable in the milk up to 36 hours after initial dosing. There was a strong correlation between maternal plasma and milk oxycodone levels within the first 24 hours, with the milk concentrations 3.2 to 3.4 times higher than serum (Seaton et al., 2007). All the infants except one had undetectable oxycodone levels in their plasma. However, neonatal outcome following oxycodone exposure via human milk was not evaluated in this study. The authors hypothesized that oxycodone may accumulate in the milk of those mothers with repeated and prolonged use (Seaton et al., 2007). Since the clearance rate of oxycodone is significantly reduced in neonates compared to older children and adults, oxycodone may accumulate in the neonate with continuous oxycodone use during breastfeeding (Pokela et al., 2005).

A case report describing a breastfed neonate experiencing oxycodone intoxication from maternal use of oxycodone was published in 2013 (Timm, 2013). Briefly, the full term infant was breastfed by a mother prescribed an oxycodone-containing medication for pain control. The baby was exclusively breastfed for two days and was admitted to the emergency department with the presentation of pinpoint pupils, hypothermia and lethargy (Timm, 2013). Given these findings were consistent with opioid intoxication, a single dose of naloxone was given intramuscularly to the neonate. Within two minutes, the baby woke up and remained alert over the next few hours.

This highlights the fact that little is known about the neonatal safety of oxycodone use during breastfeeding. Studies to date only measured oxycodone levels in the milk and plasma of
mothers and neonates up to the first 72 hours postpartum but did not clinically evaluate the
mother-infant pair for signs of adverse events (Seaton et al., 2007; Sulton-Villavasso et al.,
2012). Oxycodone may not be safer for the mother-infant pair especially for the rare CYP2D6
UM mother. No studies have investigated the role of genetic variation in the oxycodone
pathway in predicting oxycodone toxicity. Identification of genetic markers and clinical factors
may provide predictive assessment of oxycodone toxicity in this highly susceptible population.

1.5 The Effects of Ontogeny on the Safety of Opioids in Neonates

1.5.1 The Blood Brain Barrier: Structure, Function and Importance

The brain is partially protected against potentially toxic substances by two barrier
interfaces: the BBB and the blood-cerebrospinal fluid barrier (BCSFB). Ehrlich first described the
existence of these barriers in 1885. He infused Evan’s blue dye intravenously into the rat and
noticed that all the organs stained blue except the brain. However, he misinterpreted his
observation and concluded that the brain is made up of tissue with low affinity for the dye
compared to the other organs in the body (Ehrlich, 1885). Twenty years later, Ehrlich’s graduate
student, Goldmann injected Evan’s dye into the CFS and noticed that only the brain was stained
(Goldmann, 1913). Both the BBB and the BCSFB in the choroid plexus regulate the transport of
endogenous substances (i.e., ions, glucose, amino acids, red blood cells and leukocytes) and
exogenous substances (i.e., drugs) into the brain (Davson, 1976; Graff and Pollack, 2004;
Oldendorf, 1973; Saunders et al., 2008; Yudilevich and De Rose, 1971). This ensures that the
microenvironment of the brain is constantly maintained at homeostasis.

Since the surface area of the BBB is approximately 5000-fold greater than the BCSFB, it
plays the most important role in impeding the movement of neurotoxins into the brain
(Kusuhara and Sugiyama, 2001). The BBB is composed of a monolayer of brain capillary
endothelial cells (BCEC) surrounded by a basal membrane and astrocytic perivascular end-feet.
Tight junctions between adjacent BCEC restrict the paracellular movement of water-soluble
compounds into the brain. The astrocytic perivascular end-feet tightly surround the vessel wall
to maintain the integrity of the BBB. Very few molecules in the systemic circulation can gain
access to the brain with ease. Small lipid-soluble molecules (less than 400 Da) with very few
hydrogen bonds can passively diffuse across the BBB (Pardridge, 2007). BCEC contain specific carrier-mediated transporters that control the transport of essential nutrients such as hexoses (glucose, galactose), amino acids, electrolytes, nucleosides, and vitamins into the brain for normal neural function (Deeken and Loscher, 2007; Hawkins et al., 2006; Lee et al., 2001; Ohtsuki and Terasaki, 2007; Simpson et al., 2007).

Members of the large ATP-binding cassette (ABC) family of transporter proteins are efflux transporters that are expressed on the luminal membranes of BCEC (Loscher and Potschka, 2005). Some of the transporters from this family include P-gp, the breast cancer resistance protein (BCRP), and the multidrug resistance-associated proteins (MRPs). These efflux transporters are multidomain integral membrane proteins that use energy derived from ATP hydrolysis to extrude compounds from cells expressing them. Many of them have a broad substrate specificity that includes cancer chemotherapeutics, opioid analgesics, anti-epileptics, HIV-protease inhibitors, immunosuppressants, and anti-emetics (Loscher and Potschka, 2005; Schinkel and Jonker, 2003). Activity of these transporters impedes the accumulation of these neurotoxins in the brain. Dysfunction of the efflux transporters at the BBB have been associated with many neurological diseases such as Alzheimer’s disease (Bartels, 2011; Gosselet et al., 2011), amyotrophic lateral sclerosis (Garbuzova-Davis et al., 2007; Jablonski et al., 2012), Parkinson’s disease (Bartels, 2011; Marzolini et al., 2004) and multiple sclerosis (Minagar et al., 2006).

1.5.2 Opioids as Substrates for P-glycoprotein

P-gp is a 170 kDa N-glycosylated membrane protein consisting of two similar halves, each containing six putative transmembrane segments and an intracellular ATP-binding site (Schinkel, 1999). Apart from being expressed in the BBB and BCEC, it is also expressed on the apical membrane of intestinal epithelial cells of the small and large intestine, the luminal membrane of proximal tubular epithelial cells in the kidney, the biliary canalicular membrane of hepatocytes and the apical membrane of the syncytiotrophoblasts in the placenta (Mathias et al., 2005; Schinkel, 1999; Thiebaut et al., 1987). Regardless of its localization, it is believed to serve one important function in the body, to limit the accumulation of toxic substances in the organ.
Due to its strategic location on the luminal membrane of the BCEC, it has been suggested that it may serve as a “gatekeeper” by pumping P-gp substrates that enter into the endothelial cells back into the blood (Schinkel et al., 1994). P-gp transports a wide range of structurally diverse endogenous and exogenous substances that have hydrophobic regions and are positively charged at physiological pH (Schinkel, 1999). Although the exact mechanism of P-gp-mediated transport is unclear, it has been proposed that the substrate is flipped from the inner to the outer leaflet of the plasma membrane (Ambudkar et al., 1999). Endogenous substrates such as corticosteroids (cortisol and aldosterone), bilirubin, and cytokines (IL-2, IL-4, and IFN-γ) are transported by P-gp (van Kalken et al., 1993; Drach et al., 1996; Watchko et al., 1998). Many therapeutic drugs such as cancer chemotherapeutics, analgesics, anti-epileptics, HIV-protease inhibitor, immunosuppressants, and anti-emetics are substrates for P-gp (Loscher and Potschka, 2005; Schinkel and Jonker, 2003).

Several experimental approaches have been used to elucidate the important role of P-gp in the distribution of opioids across the BBB and hence its analgesic, and adverse effects (Dagenais et al., 2004; Letrent et al., 1999; Thompson et al., 2000; Zong and Pollack, 2000). Studies in mdr1a/b knockout mice have demonstrated increased the brain uptake of morphine (Dagenais et al., 2004; Thompson et al., 2000; Zong and Pollack, 2000), oxycodone (Hassan et al., 2007), and M6G (Thompson et al., 2000) were significantly increased in P-gp knockout mice compared to wild-type mice, suggesting that these opioids are substrates of P-gp. Consistent with mdr1a gene-deficient studies, animals pretreated with a P-gp inhibitor (PSC833 and GF120918) had a significantly higher opioid brain tissue-to-serum ratio compared to those not exposed to a P-gp inhibitor (Bostrom et al., 2005; Letrent et al., 1999). Furthermore, as a result of enhanced entry of opioid into the brain, mdr1a knockout mice experienced enhanced and prolonged analgesic effects when given morphine (Zong and Pollack, 2000) and oxycodone (Bostrom et al., 2005).

1.5.3 The Ontogeny of P-glycoprotein in the Blood Brain Barrier

The developing brain is vulnerable to the effects of drugs during pregnancy and at birth. Developmental expression of P-gp may contribute to variability in the disposition of opioids at
various ages of life. The expression of P-gp is relatively low in early gestation and progressively increases with advancing gestational age. The \textit{mdr1a} gene was expressed as early as embryonic day 10.5 in the mouse (Qin and Sato, 1995). The onset of \textit{mdr1a} gene expression coincided with the appearance of endothelial cell differentiation, suggesting that P-gp was the earliest marker for endothelial cell differentiation during BBB development (Qin and Sato, 1995). P-gp gene expression in the mouse brain was detected in quantitative studies at low levels during late gestation and in the newborn period (Ek et al., 2010; Qin and Sato, 1995; Tsai et al., 2002). There was significant postnatal maturation as mouse P-gp protein levels reached adult levels by three weeks of life (Tsai et al., 2002). Furthermore, immunohistochemistry studies demonstrated that mouse P-gp is exclusively localized in blood vessels (Ek et al., 2010; Matsuoka et al., 1999). Increasing P-gp immunoreactivity was detected in a greater proportion of blood vessels with progressive maturation of the mouse brain (Ek et al., 2010).

There are limited studies on the ontogeny, regional and cellular localization of P-gp in human brains. Consistent with the animal studies, immunohistochemical studies in human infants have demonstrated that P-gp, the earliest marker for brain endothelial cell differentiation during BBB development, was detected as early as 8 to 12 weeks of gestation (Schumacher and Mollgard, 1997; Virgintino et al., 2008). P-gp immunostaining was detected in human brain endothelial cells as early as 8 weeks of gestation with its prevalence and intensity progressively increasing with maturation (Daood et al., 2008; Virgintino et al., 2008). Although P-gp was detected in all the brain regions of interest (brain stem (medulla, pons, and midbrain), thalamus, cortex, hippocampus, cerebellum, and choroid plexus) (Daood et al., 2008), the immunostaining intensity was low compared to adults even at term (42 weeks of gestation), suggesting that P-gp may exhibit postnatal maturation. The ontogeny of P-gp expression in infants has not been previously studied.

1.5.4 Neonatal Sensitivity to the Central Effects of Opioids

Neonates have been shown to have heightened sensitivity to the central depressive effects of opioids compared to older infants and adults in both animal and human studies (Bouwmeester et al., 2003; Bragg et al., 1995; Koren et al., 1985; Kupferberg and Leong Way, 1963; Rai et al., 2005; Way et al., 1965). This may be attributed to differences in P-gp
expression at the BBB during postnatal development compared to adults. Lower expression of P-gp during the neonatal period is likely to influence the brain permeability to opioids and thereby increases the likelihood of opioid accumulation in the CNS. When the same dose based on weight was given, 16-day-old rats had higher morphine brain concentrations compared to 32-day-old animals, which can be primarily explained by the change in permeability of the brain to morphine (Kupferberg and Leong Way, 1963). In neonatal dogs, the dose of morphine needed to depress ventilation increases with age of the pups, suggesting that younger pups are more sensitive to the respiratory effects of morphine compared to older dogs (Bragg et al., 1995). In neonatal pigs, the clearance of morphine from the CFS at 1 week was lower than animals aged 6 weeks (Rai et al., 2005). Younger pigs had significantly higher morphine concentrations compared to those who were older (Rai et al., 2005).

There are scarce data regarding age-related differences in morphine requirements in human newborns. Full-term neonates aged 7 days or younger required significantly lower morphine doses for pain management compared to older infants (Bouwmeester et al., 2003), and younger infants were more sensitive to morphine-induced respiratory depression compared adults (Koren et al., 1985; Way et al., 1965). When the morphine dose was reduced to one-third of that given to adults, newborn infants experienced the same degree of respiratory depression as seen in adults (Way et al., 1965). The limited expression of P-gp at the BBB may allow morphine to concentrate in the brain and thereby to increase the risk for respiratory depression.

1.5.5 The Ontogeny of Drug Metabolizing Enzymes

Developmentally regulated expression of drug metabolizing enzymes such as CYP2D6, CYP3A4 and UGT2B7 has been reported (Blake et al., 2007; Choonara et al., 1989; Hines and McCarver, 2002). Substantial differences in metabolism and the elimination of opioids in the neonate can lead to differences in therapeutic efficacy and susceptibility to opioid-induced toxicity. The CYP3A subfamily is the most abundant (accounting for 30 to 40% of the total CYP content in the adult liver) and clinically important CYP enzyme in the liver. It is responsible for metabolizing approximately 50% of the drugs on the market (Johnson, 2003). CYP3A7 expression is highly expressed in the fetal liver (Hines and McCarver, 2002; Schuetz et al., 1994;
Yang et al., 1994). Fetal hepatic CYP3A7 was detected as early as 50 to 60 days of gestation and progressively increased throughout pregnancy, peaking at the first week after birth (Lacroix et al., 1997; Yang et al., 1994). However, levels of hepatic CYP3A7 at one year of life were only 10% of that in the newborn (Lacroix et al., 1997). Coincidentally, hepatic CYP3A4/5 expression begins to increase one week after birth and reaches 50% of adult levels between 6 to 12 months of age (de Wildt et al., 1999a; Lacroix et al., 1997).

The mechanism regulating fetal CYP3A7 expression and the transition from fetal isoform CYP3A7 to adult isoform CYP3A4 remains unknown. Several transcription factors such as nuclear factor I and nuclear factor κB (Saito et al., 2001) have been identified to be involved in the regulation of CYP3A7 expression. Recently, the glucocorticoid receptor (GR) has been identified to play a role in the regulation of CYP3A7 expression (Pang et al., 2012). It has been proposed that the transcription factors and/or GR bind onto the CYP3A7 5′-flanking region to activate transcription. Future studies are needed to elucidate the mechanisms regulating the developmental expression of CYP3A7 and CYP3A4.

CYP2D6 expression and activity is also subjected to marked developmental changes (Treluyer et al., 1991). CYP2D6 expression is absent in the fetal liver (Blake et al., 2005; Hines and McCarver, 2002; Ladona et al., 1991). Fetal livers ranging from 18 to 27 weeks of gestation failed to catalyze the O-demethylation of codeine and dextromethorphan to morphine and dextrorphan, respectively (Ladona et al., 1991). In addition, CYP2D6 immunoreactive protein was not detected in fetal livers aged 11 to 13 weeks gestation until 7 days of age (Shimada et al., 1996). The amount of CYP2D6 protein and activity increases progressively after birth and reached adult levels by 2 weeks of life (Blake et al., 2007). It is well known that CYP2D6 expression and activity is subjected to genetic polymorphisms. The impact of CYP2D6 genetic polymorphisms on the age-dependent expression of CYP2D6 remains unknown. Blake and colleagues assessed CYP2D6 activity using dextromethorphan as a probe substrate and found that there was concordance between CYP2D6 genotype and activity by 2 weeks of age regardless of gestational age (Blake et al., 2007). This suggests that CYP2D6 expression may be triggered by birth-related events. More importantly, during the first two weeks of life when the CYP2D6 activity is low independent of its genotype, all neonates are phenotypically PMs.
Developmental changes in UGT activity have been shown to alter the metabolism and clearance of drugs (de Wildt et al., 1999b). Specifically, the influence of developmental expression in UGT2B7 on the variation in morphine clearance has been extensively studied (Blake et al., 2005; Choonara et al., 1989; de Wildt et al., 1999b; Knibbe et al., 2009). The rate of morphine glucuronidation in fetal microsomes aged 15 to 27 weeks gestation was about 10 to 20% of that observed in adult microsomes (Pacifici et al., 1982; Pacifici et al., 1989). There was no correlation between gestational age and the rate of morphine glucuronidation, which suggests that the UGT2B7 activity may be regulated by the birthing process (Pacifici et al., 1982). The mean plasma clearance of morphine in premature infants (aged 24 to 37 weeks gestation, postnatal age 2 to 12 days) was 5-fold lower compared to children 1 to 16 years of age and reached adults levels by 2 to 6 months of life (Choonara et al., 1989). Even when adjusted for body weight, younger neonates (less than postnatal day 10) had a significantly lower capacity to glucuronidate morphine compared to older neonates (older than postnatal day 10), which resulted in higher plasma morphine levels (Knibbe et al., 2009). Since the glucuronidation pathway is the primary metabolic pathway for other opioids such as oxymorphone and hydromorphone, the ontogeny of UGT2B7 may have a similar influence on the clearance of these opioids. Enhanced CNS opioid exposure may be related to developmental differences in P-gp expression in the brain and hepatic drug-metabolizing enzymes and thereby heightens the neonate’s sensitivity to opioids compared to older infants.

2 Thesis Scope

2.1 Statement of the Problem and Overall Aim

Opioids have been used for thousands of years and are a valuable tool for the treatment of pain. However, the efficacy and safety of opioids are characterized by great interindividual variability. Of great concern is the use of opioids in highly susceptible populations, such as infants and their mothers. Physicians are now resorting to prescribing other opioid analgesics such as oxycodone due to the increasing number of fatal or life threatening reports of codeine intoxication in young children as well as adults (Ciszkowski et al., 2009; Gasche et al., 2004; Koren et al., 2006; Madadi et al., 2009b).
Several important knowledge gaps need to be addressed in order to minimize the risk of life-threatening CNS depression following opioid administration in these vulnerable populations. Firstly, although breastfeeding mothers are sometimes prescribed oxycodone instead of codeine for the management of postpartum pain in the hope of circumventing the risk for neonatal codeine intoxication, it is unknown whether this switch reduces the risk for opioid intoxication. Secondly, since oxycodone’s efficacy and safety may be under genetic influences, it is important to explore the potential utility of maternal genetic variants in predicting oxycodone toxicity in the mother-infant pair. Thirdly, although neonates have been shown to have heighten sensitivity to the effects of opioids, to date, the mechanism underlying this phenomenon is unknown. Thus, it is important to explore the contribution of age-related dynamic changes at the BBB in order to better understand the pharmacodynamic effect of opioids. Lastly, although patients using opioids are often prescribed other concomitant drugs, it is unknown whether these combinations can lead to detrimental outcomes. Thus, the overall aim of this thesis is to investigate the contribution of genetic polymorphisms, ontogeny and drug interactions in opioid-induced toxicity in order to address the knowledge gaps outlined above. We hypothesize that this research will reveal some important predictive markers associated with opioid-induced toxicity, and that this new knowledge will increase both the safety and effectiveness of opioids in clinical practice.

2.2 Objectives and Hypotheses

This thesis is divided into four separate studies, which were guided by the four primary objectives and hypotheses outlined below. These studies are presented in detail in their respective chapters.

**PRIMARY OBJECTIVES:**

1. To determine if the replacement of codeine by oxycodone is safe for both the mother and infant by quantifying the incidence of CNS depression in neonates reported by mothers taking oxycodone during breastfeeding compared to infants breastfed by codeine-medicated or acetaminophen-medicated mothers during breastfeeding (Chapter 2).
Both the FDA and Health Canada issued public warnings about the safety of codeine use during breastfeeding in response to the fatal report of a 13-day old infant whose codeine-prescribed mother was a CYP2D6 UM (Health Canada, 2008; Koren et al., 2006; U.S Food and Drug Administration (FDA), 2007). Some physicians started prescribing oxycodone as an alternative to codeine for moderate pain relief in the postpartum period without concrete evidence to support this decision. This study aims to assess the effect of oxycodone exposure via breast milk in infants by quantifying the incidence of neonatal CNS depression according to maternal report. The results are meant to determine whether oxycodone is a safe alternative for codeine and to identify important features of this potentially fatal adverse reaction so clinical approaches can be developed to prevent neonatal opioid toxicity.

II. To determine the contribution of maternal genetic polymorphisms in drug metabolizing enzymes, transporters and receptors involved in the metabolism and response of oxycodone with respect to maternal and neonatal CNS depression following oxycodone use during breastfeeding (Chapter 3).

Maternal genetic variants involved in the metabolism, transport and response in the opioid pathway have been identified to predict neonatal and maternal codeine-induced toxicity (Madadi et al., 2009b; Sistonen et al., 2007). While the analgesic effect of oxycodone is less dependent on CYP2D6 activity than it is for codeine, the contribution of the CYP2D6 genotype in predicting efficacy and avoiding toxicity in breastfeeding mothers is unknown. This study aims to investigate the role of maternal genetic variants in CYP2D6, CYP3A5, ABCB1 and/or OPRM1 in oxycodone toxicity in mothers and infants. It is expected that determination of whether these maternal genetic markers affect oxycodone toxicity in these mothers and their infants will improve the safety and effective use of oxycodone in the highly susceptible infant population.

III. To determine the ontogeny of P-gp in endothelial cells of the developing BBB in order to understand why young infants are sensitive to the central effects of morphine during the first months of life compared to older infants and adults (Chapter 4).
The efflux transporter, P-gp expressed at the BBB serves as a “gatekeeper” in order to prevent the accumulation of morphine within the brain. It is well established that young infants have a heightened sensitivity to the central depressive effect of morphine, possibly attributed to the limited expression of P-gp at the BBB. This study aimed to investigate the developmental expression of P-gp in endothelial cells of the developing human BBB. The results will shed some light on the mechanism of neonatal drug exposure in the brain that may be associated with life threatening adverse events. The implication of these findings is not restricted to morphine, but other commonly used pediatric drugs, which are also substrates for P-gp.

IV. To assess the relationship between genetic polymorphisms and drug interactions as related to codeine and morphine concentrations (Chapter 5).

Both genetic polymorphisms and drug interactions can influence the efficacy and safety of codeine. It is well known that concomitant use of opioids with benzodiazepines, hypnotics, and/or alcohol, may enhance the depressive effect of opioids on respiratory drive (Haberman et al., 1995; Leander and Lucot, 1977; Ruttenber et al., 1990). Despite the known risks associated with concurrent use of opioids with non-opioid CNS depressants, it is difficult to document the toxicological effects of these drug interactions in human studies. In the absence of controlled-clinical trial data, toxicological data from post-mortem investigations are valuable, as they assist in the interpretation of drug concentrations in deaths involving opioid intoxication. This study aimed to investigate the influence of genetic variation in the codeine and morphine metabolic pathways and drug interactions on codeine and morphine concentrations in codeine-related deaths in Ontario, Canada. The results from this unique and powerful cohort of deceased individuals have the potential to influence prescribing-decisions and will also assist in the interpretation of drug concentration in deaths involving codeine intoxication.

PRIMARY HYPOTHESES AND RATIONALE:

1. The incidence of neonatal CNS depression reported by oxycodone-medicated mothers will be similar to those reported by codeine-medicated mothers, but higher than those reported by acetaminophen-medicated mothers.
Rationale: Since oxycodone is an opioid, it is expected to have a similar depressive effect on the nursing infant as codeine. Acetaminophen is a mild anti-inflammatory agent that does not have any sedating properties and therefore not expected to have any depressive effect on the nursing infant. If the incidence of neonatal CNS depression in the oxycodone cohort is similar to that of the codeine cohort, then it demonstrates that oxycodone cannot be assumed to be a safe alternative for codeine during the postpartum period.

II. Neonatal CNS depression following oxycodone exposure via breast milk is associated with maternal genetic polymorphisms in *CYP2D6*, *CYP3A5*, *ABCB1* and *OPRM1*; and maternal genetic polymorphisms will be associated with maternal CNS depression following oxycodone use.

Rationale: Since genetic polymorphisms in drug metabolizing enzymes, receptor and transporters influence the pharmacokinetics and pharmacodynamics of oxycodone, these polymorphisms are likely going to predict adverse effects following oxycodone use in the mother-infant pair. CYP2D6 UM mothers are expected to produce more oxymorphone compared to the other genotypes. In this case, more oxymorphone may be excreted into breast milk leading to toxicity in the breastfed infant. Mothers with reduced P-gp and MOR function will be protected against oxycodone-induced toxicity compared to those with normal function.

III. The expression of P-gp in the developing human BBB will be developmentally regulated such that its expression will be limited at birth, but will increase significantly with postnatal maturation.

Rationale: Since some studies have suggested that BBB P-gp expression is low during the fetal period, it is likely that its expression at birth is low as well, which allows opioids to concentrate in the brain. With increasing P-gp expression in the neonatal period, older infants have the capacity to efflux opioid from the brain before it has a chance to exert its toxic effect.

IV. Genetic polymorphisms and drug interactions will result in discordance between the CYP2D6 genotype and phenotype.
Rationale: There are numerous drugs on the market known to interact with opioids, leading to unreliable CYP2D6 genotype-to-phenotype predictions. Since codeine is a CYP2D6 substrate, its metabolism will be inhibited by concomitant use of a CYP2D6 inhibitor. The potency of the CYP2D6 inhibitor affects the degree of inhibition of CYP2D6 activity. Concomitant use of a strong CYP2D6 inhibitor is expected to significantly decrease the morphine-to-codeine ratio compared to the presence of a weak CYP2D6 inhibitor.

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Chapter 2 Central Nervous System Depression of Neonates Breastfed by Mothers Receiving Oxycodone for Postpartum Analgesia


[JL collected the data, performed data analysis, and prepared the manuscript for submission].
1 Abstract

Objective: To quantify the incidence of central nervous system (CNS) depression in neonates breastfed by oxycodone-medicated mothers, as compared to neonates whose breastfeeding mothers used 1) codeine, or 2) acetaminophen-only. Study Design: A retrospective study consisting of 3 cohorts in 533 breastfeeding mother-infant pairs exposed to oxycodone (n=139), codeine (n=210) or acetaminophen-only (n=184) was conducted. Standardized questionnaires were administered during the post-partum period to elucidate maternal and neonatal health outcomes temporally related to analgesia exposure based on maternal reports. Results: Maternal exposure to oxycodone during breastfeeding was associated with a 20.1% rate of infant CNS depression (28/139) compared to 0.5% in the acetaminophen group (1/184) [p<0.0001, OR 46.16 95% CI 6.2-344.2] and 16.7% (35/210) in the codeine group [p>0.05, OR 0.79 95% CI 0.46-1.38]. Mothers of symptomatic neonates in the oxycodone and codeine cohorts took significantly higher doses of medication compared to mothers of asymptomatic infants in the same cohorts [p=0.0005 oxycodone (median 0.4 (0.03-4.06) vs. median 0.15 (0.02-2.25) mg/kg/day; codeine p<0.001 median 1.4 (0.7-10.5) vs. 0.9 (0.18-5.8) mg/kg/day]. Mothers were significantly more likely to experience sedative adverse effects from oxycodone as compared to codeine [p<0.0001, OR 17.62 95% CI 9.95-31.21]. Conclusion: Oxycodone is not a safer alternative than codeine in breastfed infants.
2 Introduction

Oxycodone is a semisynthetic opioid commonly used to treat moderate to severe pain postoperatively and in cancer. There is limited information on the excretion of oxycodone into breast milk and the subsequent effects, if any, on the breastfed child (Seaton et al., 2007). Based on a few characteristics of oxycodone, it has been suggested that it may be readily transferred into breast milk (Seaton et al., 2007; Ito, 2000). Oxycodone has high oral bioavailability (60-87% in adults) and rapid oral absorption (Micromedex, 2006; Purdue Pharma Std., 2009). It is moderately protein bound (38-45%) such that there may be a sufficient amount of unbound drug in the maternal plasma to be transferred into milk (Poyhia et al., 1994). Oxycodone is a weak base (pKa 8.5) (Atkinson et al., 1988). Since breast milk (pH 7.2) is slightly more acidic than plasma (pH 7.4), unbound oxycodone can be subjected to an “ion-trapping” effect.

Due to the recent publicity regarding neonatal central nervous system (CNS) depression following codeine and breastfeeding (Koren et al., 2006), some clinicians are now prescribing oxycodone in place of codeine during the postpartum period. However, like codeine, oxycodone is a substrate for the cytochrome P450 (CYP) 2D6 and 3A4. It has been demonstrated that CYP3A4 produces the major metabolite, noroxycodone via N-demethylation while CYP2D6 catalyzes O-demethylation producing oxymorphone, which accounts for 10% of the circulating oxycodone metabolites. These metabolites have varying potencies and affinities for the mu opioid receptor (MOR). Oxymorphone is 14 times more potent than oxycodone (Chen et al., 1991). Its affinity for the MOR is 40 and threefold higher than oxycodone and morphine respectively.

The neonatal safety of oxycodone use during breastfeeding has not been established. Thus it is important to clarify the incidence of neonatal CNS depression. The objectives of this study were to quantify the incidence of CNS depression in neonates breastfed by oxycodone-medicated mothers, to determine if oxycodone is a safer alternative in place of codeine, and to identify characteristics of symptomatic cases that may help with clinical management.
3 Subjects and Methods

A retrospective study consisting of three cohorts [breastfeeding mother-infant pairs exposed to oxycodone, codeine, or acetaminophen-only] was conducted, after obtaining Research Ethics Board approval from the Hospital for Sick Children (Toronto, Canada). The mother-infant pairs were recruited from the Motherisk Program at the Hospital for Sick Children, a Teratology Information Center that counsels women using evidence-based information regarding the safety of using medication during pregnancy and breastfeeding.

The files of women who had called to inquire about the safety of acetaminophen-only, codeine or oxycodone during breastfeeding were reviewed.

The acetaminophen-only cohort (n= 590) consisted of mothers who had contacted the Motherisk Program between January 2004 and December 2008 to inquire about the safety of acetaminophen during breastfeeding. In the codeine cohort (n= 681), mothers had contacted Motherisk inquiring about codeine with acetaminophen (for example, Tylenol with codeine T1: acetaminophen 300mg + codeine phosphate 8mg; T2: acetaminophen 300mg + codeine phosphate 15mg; or most commonly T3: acetaminophen 300mg + codeine phosphate 30mg) or codeine alone between January 2004 and December 2008. A subset of the codeine study participants was included in a previous study by Madadi et al. 2009 (Madadi et al., 2009). The oxycodone cohort (n= 289), recruited between January 2007 and October 2010 as a response to the increased prevalence of oxycodone use in the breastfeeding population, consisted of mothers who had about oxycodone-acetaminophen (for example Percocet®: acetaminophen 325mg + 5mg oxycodone or 10mg oxycodone or 20mg oxycodone) or oxycodone alone during breastfeeding.

Original intake forms from the Motherisk Program contain maternal demographic information (age, parity, gravidity, and weight), infant information (gender, birth weight, birth defects, postmenstrual age (PMA, gestational age + chronological age) of child at time of opioid/acetaminophen use), dose of oxycodone/codeine/acetaminophen that had been
prescribed, indication, frequency of breastfeeding, and duration of breastfeeding recorded at the time of consultation.

After informed maternal consent, a standardized follow-up questionnaire was administered during a telephone interview to elucidate adverse maternal and neonatal events with the focus on CNS depression temporally related to any of the three of the drugs according to maternal self-reports, and the reversibility of CNS depression upon discontinuation of opioid usage or breastfeeding. Infants identified with symptoms of CNS depression (sleepiness/lethargy or not waking up for feeding during the time period of drug exposure via breast milk) were classified as “symptomatic”. Dose of medication used, frequency of breastfeeding, duration of breastfeeding, supplementation with formula, maternal ethnicity, and maternal side effects during medication use were also recorded during this second consultation. Mothers identified as experiencing sedation were also classified as “symptomatic”.

We excluded mothers who did not provide consent for the telephone interview, mothers who took other sedative medications beside oxycodone or codeine alone (such as benzodiazepines, skeletal muscle relaxant, and oxycodone and codeine concurrently) and mothers who reported using alcohol or drugs of abuse in late pregnancy or while breastfeeding. We also excluded cases where infants were diagnosed with CNS anomalies.
4 Results

We examined the files of 1560 women inquiring about oxycodone (n=289), codeine (n=681), or acetaminophen-only (n=590). Of these, a total of 533 women were available for follow up (n=139 in the oxycodone cohort, n=210 in the codeine cohort and n=184 in the acetaminophen-only group) (Figure 2.1 for exclusion criteria).

![Figure 2.1 Reasons for excluding patients who had inquired about acetaminophen-only, oxycodone or codeine.](image)

In the codeine group, 65% (137/210) of mothers were taking a combination codeine-acetaminophen product, while the rest were taking codeine alone (73/210). In the oxycodone group, 52.5% (73/139) of mothers were taking a combination oxycodone-acetaminophen product, while the rest were taking oxycodone alone (66/139).
There were no differences in maternal age, ethnic distribution, and infant birth weight among the three cohorts (Table 2.1). However, maternal weight, parity, and postmenstrual age of infant (PMA) at the time of drug exposure were significantly different among the three cohorts. Further statistical analysis between the oxycodone and codeine cohorts only showed that PMA and parity remained significant (Table 2.2).

Table 2.1 Demographic characteristics of mothers and their infants in the oxycodone, codeine, and acetaminophen-only cohort.

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone cohort (n=139)</th>
<th>Codeine cohort (n=210)</th>
<th>Acetaminophen only (n=184)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.90 (4.64)</td>
<td>32.65 (4.51)</td>
<td>31.95 (4.97)</td>
<td>0.18</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>73.20 (14.28)</td>
<td>72.91 (14.74)</td>
<td>67.85 (13.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nulliparous (%)</td>
<td>51 (37%)</td>
<td>107 (60%)</td>
<td>109 (61%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maternal non-Caucasian ethnicity no. (%)</td>
<td>17 (28.7%)</td>
<td>31 (38.3%)</td>
<td>46 (25.0%)</td>
<td>0.58 a</td>
</tr>
<tr>
<td>Infant birth weight (kg)</td>
<td>3.37 (0.59)</td>
<td>3.42 (0.59)</td>
<td>3.41 (0.58)</td>
<td>0.76</td>
</tr>
<tr>
<td>Infant PMA (weeks) b</td>
<td>53.6 (32.9)</td>
<td>53.9 (16.4)</td>
<td>53.9 (13.3)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

n_{dcr}, number of responses per descriptor

Maternal-infant pair characteristics, mean (SD)

a For dichotomous variables, Fisher’s Exact test was used to compare the contingencies, p<0.05 denotes statistical significance

b Age of infants at the time of oxycodone/codeine/acetaminophen-only exposure and breastfeeding
### Table 2.2 Demographic characteristics of mothers and neonates in oxycodone and codeine cohort.

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone cohort (n=139)</th>
<th>Codeine cohort (n=210)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.90 (4.64)</td>
<td>32.65 (4.51)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 139</td>
<td>n_dcr= 153</td>
<td></td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>73.20 (14.28)</td>
<td>72.91 (14.74)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 139</td>
<td>n_dcr= 183</td>
<td></td>
</tr>
<tr>
<td>Nulliparous (%)</td>
<td>51 (37%)</td>
<td>107(60%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 139</td>
<td>n_dcr= 179</td>
<td></td>
</tr>
<tr>
<td>Maternal non-Caucasian ethnicity no. (%)</td>
<td>17 (28.7%)</td>
<td>31 (38.3%)</td>
<td>0.58 a</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 139</td>
<td>n_dcr= 210</td>
<td></td>
</tr>
<tr>
<td>Infant birth weight (kg)</td>
<td>3.37 (0.59)</td>
<td>3.42 (0.59)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 139</td>
<td>n_dcr= 207</td>
<td></td>
</tr>
<tr>
<td>Infant PMA (weeks)</td>
<td>53.6 (32.9)</td>
<td>53.9 (16.4)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>n_dcr= 138</td>
<td>n_dcr= 165</td>
<td></td>
</tr>
</tbody>
</table>

n_dcr, number of responses per descriptor

Maternal-infant pair characteristics, mean (SD)

a For dichotomous variables, Fisher’s Exact test was used to compare the contingencies, p< 0.05 denotes statistical significance
The indication for oxycodone, codeine, and acetaminophen-only use during breastfeeding were significantly different amongst the three cohorts (Table 2.3), but not between the oxycodone and codeine cohorts (Table 2.4).

Table 2.3 Maternal indication for oxycodone, codeine, or acetaminophen-only.

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone (^1)</th>
<th>Codeine (^2)</th>
<th>Acetaminophen only</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n_{dcr} = 139)</td>
<td>(n_{dcr} = 210)</td>
<td>(n_{dcr} = 184)</td>
<td></td>
</tr>
<tr>
<td>Caesarian Section</td>
<td>30</td>
<td>60</td>
<td>9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vaginal/Episiotomy</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>0.0673</td>
</tr>
<tr>
<td>Headache/migraine</td>
<td>6</td>
<td>14</td>
<td>62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dental/minor surgery</td>
<td>38</td>
<td>65</td>
<td>8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.614</td>
</tr>
<tr>
<td>Cold/cold-related pain</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>37</td>
<td>45</td>
<td>24</td>
<td>0.1046</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>9</td>
<td>14</td>
<td>0.3719</td>
</tr>
</tbody>
</table>

\(n_{dcr}\), number of responses per descriptor

\(^1\) 52.5\% (73/139) of mothers were prescribed a combination oxycodone-acetaminophen product.

\(^2\) 65\% (137/210) of mothers were prescribed a combination codeine-acetaminophen product.
Table 2.4 Indication for using oxycodone and codeine.

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone cohort</th>
<th>Codeine cohort</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n_{dcr}=139$</td>
<td>$n_{dcr}=210$</td>
<td></td>
</tr>
<tr>
<td>Caesarian Section</td>
<td>30</td>
<td>60</td>
<td>0.17</td>
</tr>
<tr>
<td>Vaginal/Episiotomy</td>
<td>1</td>
<td>7</td>
<td>0.15</td>
</tr>
<tr>
<td>Headache/migraine</td>
<td>6</td>
<td>14</td>
<td>0.48</td>
</tr>
<tr>
<td>Dental/minor surgery</td>
<td>38</td>
<td>65</td>
<td>0.55</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>10</td>
<td>10</td>
<td>0.36</td>
</tr>
<tr>
<td>Other</td>
<td>37</td>
<td>45</td>
<td>0.30</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>9</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$n_{dcr}$, number of responses per descriptor

In the oxycodone cohort, 20.1% (28/139) of mothers reported neonatal CNS depression compared to only 0.5% (1/184) of mothers in the acetaminophen-only group [$p<0.0001$, OR 46.16, 95% CI 6.191-344.2] and 16.7% (35/210) of mothers in the codeine group [$p>0.05$ OR 0.7929, 95% CI 0.4570-1.376]. Out of the 28 neonates who experienced sedation after being breastfed by an oxycodone-medicated mother, 4 infants were also observed to have “irregular breathing”. In addition to death as a result of opioid toxicity in one infant whose mother was using codeine (Koren et al., 2006; Madadi et al., 2009), 4 infants in the codeine cohort were taken to the emergency room for symptoms of lethargy.

Oxycodone-medicated mothers of symptomatic infants took significantly higher doses of oxycodone than mothers of asymptomatic infants (median 0.4 (0.03-4.060) mg/kg/day ($p=0.005$) vs. median 0.15 (0.02-2.250) mg/kg/day). However, all mothers received doses that fell within the recommended range (not exceeding 40mg/d) (Table 2.5 for recommended
dosing regimens). Symptomatic infants breastfed by oxycodone-medicated mothers had significantly longer consecutive hours of uninterrupted sleep per day than asymptomatic infants (median 5 (3-24) hours/day ($p=0.0216$) vs. median 4 (1-21) hours/day). Similarly, among the cohort receiving codeine, mothers of symptomatic infants took significantly higher codeine doses (median 1.4 (0.7-10.5) vs. 0.9 (0.18-5.8) mg/kg/day ($p<0.001$)) than mothers of asymptomatic infants (Table 2.6). Maternal reports of resolution of neonatal symptoms when maternal narcotics or breastfeeding ceased were also documented with 38/39 and 30/35 mothers reporting reversibility of neonatal symptoms upon cessation of oxycodone and codeine or breastfeeding respectively.

**Table 2.5 Oxycodone dosing regimen.**

<table>
<thead>
<tr>
<th></th>
<th>Naïve patients</th>
<th>Patients receiving alternative opioid</th>
<th>Use with non-opioid medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>OxyIR</td>
<td>5 or 10mg q6h</td>
<td>Determine total daily dosage of present analgesic, calculate approximate daily oral oxycodone dosage to provide equivalent analgesia $^1$</td>
<td>Non-opioid medication may be continued; if discontinue non-opioid, should increase opioid dose to compensate</td>
</tr>
<tr>
<td>OxyContin</td>
<td>10 or 20mg q12h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percocet (5mg oxycodone + 325mg acetaminophen)</td>
<td>1 tablet q6h $^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percocet-demi (2.5mg oxycodone + 325 acetaminophen)</td>
<td>1 to 2 tablets q6h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supeudol</td>
<td>5 or 10mg q6h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: individual dosing requirement vary considerably based on each patient’s age, weight, severity, cause of pain, and medical and analgesic history (CPS, 2010)

$^1$ Treat appropriate pain with only one opioid at a time

$^2$ May occasionally be necessary to exceed usual recommended dose in cases of more severe pain or tolerance
Table 2.6 Characteristics of symptomatic vs. asymptomatic mother-infant pairs within the oxycodone and codeine cohort.

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone Cohort</th>
<th>Codeine Cohort</th>
<th>P value</th>
<th>Oxycodone Cohort</th>
<th>Codeine Cohort</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic</td>
<td>Asymptomatic</td>
<td></td>
<td>Symptomatic</td>
<td>Asymptomatic</td>
<td></td>
</tr>
<tr>
<td>Maternal dose (mg/kg/day) a</td>
<td>0.4 (0.03-4.06)</td>
<td>0.15 (0.02-2.25)</td>
<td>0.001</td>
<td>1.4 (0.7-10.5)</td>
<td>0.9 (0.18-5.80)</td>
<td>0.001</td>
</tr>
<tr>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td></td>
<td>n_dcr = 25</td>
<td>n_dcr = 67</td>
<td></td>
</tr>
<tr>
<td>Infant PMA (weeks) b</td>
<td>47.6 (16.1)</td>
<td>55.2 (35.9)</td>
<td>0.456</td>
<td>52.0 (15.0)</td>
<td>54.2 (16.6)</td>
<td>0.439</td>
</tr>
<tr>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td></td>
<td>n_dcr = 35</td>
<td>n_dcr = 130</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding duration</td>
<td>17.8 (23.7)</td>
<td>11.1 (23.7)</td>
<td>0.051</td>
<td>7 (1-180)</td>
<td>4 (1-247)</td>
<td>0.12</td>
</tr>
<tr>
<td>days breastfed with concurrent</td>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td>n_dcr = 34</td>
<td>n_dcr = 157</td>
<td></td>
</tr>
<tr>
<td>maternal medication consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of breastfeeding</td>
<td>7.5 (3.0)</td>
<td>7.8 (2.7)</td>
<td>0.514</td>
<td>8 (2.88)</td>
<td>7 (2.65)</td>
<td>0.12</td>
</tr>
<tr>
<td>(number of feeds/day)</td>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td>n_dcr = 29</td>
<td>n_dcr = 166</td>
<td></td>
</tr>
<tr>
<td>Formula supplementation</td>
<td>13 (46.4%)</td>
<td>52 (46.8%)</td>
<td>0.483^c</td>
<td>20 (64.5%)</td>
<td>89 (55.6%)</td>
<td>0.298^c</td>
</tr>
<tr>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td></td>
<td>n_dcr = 31</td>
<td>n_dcr = 160</td>
<td></td>
</tr>
<tr>
<td>Hours slept by infant</td>
<td>5 (3-24)</td>
<td>4 (1-21)</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n_dcr = 28</td>
<td>n_dcr = 111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n_total, number of total responses

n_dcr, number of responses per descriptor

Maternal-infant pair characteristics, mean (SD) and median (range) as appropriate

a Maternal dose was within recommended adult dose of 5 or 10mg oxycodone q6h (CPS, 2010)

b Age of infants at the time of oxycodone/codeine/acetaminophen-only exposure and breastfeeding

c For dichotomous variables, Fisher’s Exact test was used to compare the contingencies, p < 0.05 denotes statistical significance
In fact oxycodone- and codeine-medicated mothers of symptomatic babies were significantly more likely to experience CNS depression themselves [26/28 maternal CNS depression with symptomatic infant vs. 66/111 maternal CNS depression with asymptomatic infant, *p*<0.001, OR=8.864, 95% CI 2.002-39.24 in oxycodone cohort] [15/35 maternal CNS depression with symptomatic infant vs. 6/175 maternal CNS depression with asymptomatic infant, *p*<0.001, OR=21.1 95% CI 7.4-60.6 in codeine cohort].

Oxycodone-medicated mothers were more likely to experience CNS depressive adverse effects compared to those using codeine [92/139 in oxycodone cohort vs. 21/210 in codeine cohort; *p*<0.0001, OR 17.62, 95% CI 9.95-31.21]. In addition to experiencing lethargy, a proportion of mothers in the oxycodone and codeine cohorts experienced other side effects such as nausea, vomiting, constipation, dizziness, weakness, and confusion (Table 2.7).

**Table 2.7 Maternal adverse events reported with oxycodone or codeine use during breastfeeding.**

<table>
<thead>
<tr>
<th></th>
<th>Oxycodone (n=139)</th>
<th>Codeine (n=139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedation</td>
<td>92 (%)</td>
<td>21 (%)</td>
</tr>
<tr>
<td><strong>Other concomitant adverse events</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>19 (21)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8 (8.6)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>Constipation</td>
<td>23 (25)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>23 (25)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Weakness</td>
<td>8 (8.6)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Confusion</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rash</td>
<td>0 (0)</td>
<td>2 (9.5)</td>
</tr>
</tbody>
</table>

1 Of the proportion of mothers who reported experiencing side effects with oxycodone or codeine medications, all of these mothers listed sedation as an adverse event. All other side effects with oxycodone or codeine medications occurred in conjunction with sedation. Mothers were significantly more likely to experience sedative adverse effects from oxycodone as compared to codeine [*p*< 0.0001, OR 17.62, 95% CI 9.95-31.21].
5 Discussion

Following a case of fatality in a breastfed infant exposed to codeine through breast milk (Koren et al., 2006), both the FDA and Health Canada published warnings indicating that codeine use in breastfeeding may not be safe for all infants (FDA, 2007; Health Canada, 2008). As a result, some institutions are now replacing codeine with oxycodone for postpartum pain relief. However, Seaton et al. detected oxycodone in breast milk from all mothers taking any dose of oxycodone (Seaton et al., 2007). In fact, the oxycodone breast milk levels strongly correlated with plasma levels suggesting that oxycodone persisted in the breast milk of some mothers. Therefore, it is important to address the neonatal safety of oxycodone during breastfeeding.

In this present study we compared the maternal reports of CNS depression in breastfed infants exposed to oxycodone versus infants who were exposed to codeine or acetaminophen alone. Our analysis reveals several important features of this potentially fatal adverse reaction: the maternal self-report of neonatal CNS depression is higher in neonates breastfed by oxycodone-medicated mothers as compared to those breastfed by acetaminophen-medicated mothers. Maternal oxycodone use is associated with a similar incidence of neonatal CNS depression as compared to codeine and should not be considered to be a safe alternative for codeine during breastfeeding. Symptomatic infants of oxycodone-medicated mothers were sleeping longer than asymptomatic infants. It is important to note that in most cases of CNS depression in the oxycodone and codeine cohort, the parents reported dramatic neonatal improvement when exposure of the opioid ceased. There was a dose-response relationship with mothers of symptomatic infants having consumed on average 50% more oxycodone and codeine per kg of maternal body weight. However, some mothers reported neonatal CNS depression when they were consuming as little as 0.03 mg/kg of oxycodone daily. Furthermore, there was a trend for mothers of symptomatic infants to have used oxycodone or codeine for longer periods of time than mothers of asymptomatic infants. Importantly, our findings suggest that maternal CNS depression is a strong predictor for neonatal CNS depression for both oxycodone and codeine. If clinicians observe maternal CNS depression, they need to monitor the child for it as well. Lastly, oxycodone-medicated mothers were more likely to experience
CNS depressive adverse effects in addition to other side effects known to be associated with opioid use compared to those using codeine.

Several differences between the three cohorts in this study need to be highlighted. Firstly, maternal indications for receiving acetaminophen or opioids were different amongst the cohorts. This is reflective of the general practice of prescribing opioids for pain relief following Caesarian-section or episiotomy in Canada. Therefore, questions related to comparative efficacy between codeine, oxycodone, and acetaminophen cannot be addressed by this study. Secondly, sequential statistical analysis revealed that mothers in the codeine group were significantly more likely than those in the oxycodone group to be first-time mothers. Arguably, the inexperience of first time mothers may lead to hypervigilance and increased anxiety which could translate into increased reporting of CNS depressive symptoms. Thus, while we observed a similar incidence of neonatal CNS depression between oxycodone and codeine, parity could have biased these results causing overreporting of CNS depression in the codeine group. Thirdly, infants who were exposed to oxycodone via breast milk were slightly younger in the oxycodone group as compared to the codeine and acetaminophen group. Pharmacodynamic modeling has revealed that compromised neonatal opioid clearance capacity, (which is closely related to age) may predispose infants to CNS depressive side effects when exposed to maternal opioids (Willmann et al., 2009). However, within the oxycodone group, there was no difference in postmenstrual age between symptomatic and asymptomatic infants.

The major limitation was the retrospective nature of this study and thus the potential for recall bias was introduced. Furthermore, the population of mothers interviewed were self-selected as they took the initiative to call the Motherisk Program and ask for safety advice. Due to this, it is possible that these women may have exhibited increased vigilance in monitoring their infants for symptoms of ADR than the general population, but this increased attention would also likely improve recall of the event. The control group, the acetaminophen cohort was deemed critical to account for non-specific features that may resemble neonatal CNS depression especially when they are based on maternal reports. In accordance, there is only one maternal-positive report of infant CNS-depression when a mother was breastfeeding and consuming acetaminophen alone.
In conclusion, maternal consumption of oxycodone is associated with an increased risk of CNS depression in the breastfed infant, such that 1 in 5 breastfed infants with oxycodone-mediated women experienced symptoms of CNS depression. Therefore, replacement of codeine by oxycodone during breastfeeding cannot be assumed to be safe for the child and the mother. In the future, prospective and pharmacogenetic studies are needed to investigate other factors related to maternal oxycodone use and neonatal CNS depression.
6 References


Chapter 3 Putative Association of \textit{ABCB1 2677G>T/A} with Oxycodone Induced Central Nervous System Depression in Breastfeeding Mothers


[JL collected the data, performed data analysis, and prepared the manuscript for submission. CJDR, BCC and MRH performed the genetic analysis].
1 Abstract

**Objective:** To assess the effect of maternal *CYP2D6, CYP3A5, ABCB1*, and *OPRM1* polymorphisms in predicting both neonatal and maternal central nervous system (CNS) depression after oxycodone use during lactation. **Study Design:** A nested case-control study in 67 breastfeeding mother-infant pairs exposed to oxycodone was conducted. Cases were defined as parental reports of lethargy in the infant temporally related to oxycodone exposure via breast milk. Maternal saliva samples were analyzed for 18 polymorphisms in 4 genes, *CYP2D6, CYP3A5, OPRM1, ABCB1* involved in oxycodone metabolism and response. **Results:** Mothers of symptomatic infants were using oxycodone for a longer period of time during breastfeeding compared to those of asymptomatic infants (*p*<0.0001). None of the maternal genetic variants in the 4 genes were associated with oxycodone-induced depression in neonates. However, mothers carrying at least one copy of the *ABCB1 2677 T* variant had an increased risk of experiencing sedation themselves [OR 2.35, 95% CI 1.06-5.28; *P* value=0.03]. **Conclusion:** The *ABCB1 2677 T* variant may predict oxycodone-induced CNS depression in breastfeeding mothers.
2 Introduction

Managing maternal pain following childbirth often requires the utilization of pharmacological methods such as opioid analgesics. Codeine and oxycodone are currently being utilized for moderate pain relief in the postpartum period. Due to the safety concerns related to codeine use and breastfeeding (Koren et al., 2006; Madadi et al., 2007), some institutions are now replacing codeine with oxycodone in the postpartum period. Recently, our group showed that maternal oxycodone use was associated with a similar incidence of neonatal central nervous system (CNS) depression in breastfed infants as compared with codeine (Lam et al., 2012a). Particularly, maternal oxycodone dose per kg of maternal weight, maternal duration of oxycodone use during the postpartum period, and high concordance between CNS depression in the mother and her baby were some of the clinical factors that were identified as strong predictors of neonatal CNS depression in breastfed infants (Lam et al., 2012a).

Similar to codeine, oxycodone is metabolized by the highly polymorphic enzyme, CYP2D6, which was reported to catalyze approximately 11% of the metabolism of oxycodone into active oxymorphine (Cone et al., 1983). However, significant variation in the amount of oxymorphine formed from oxycodone has been noted (Samer et al., 2010b). The N-demethylation via CYP3A, accounts for up to 80% of oxycodone oxidative metabolism, producing the major metabolite, inactive noroxycodone (Lalovic et al., 2006). Significant variation with the amount of noroxycodone formed from oxycodone has also been described (Samer et al., 2010b). These metabolites have varying potencies and affinities for the mu opioid receptor (MOR). Oxymorphine is 64 times more potent than oxycodone at the mu opioid receptor (Volpe et al., 2011). Since oxymorphine is more potent than the parent compound, it has been suggested that this metabolite is partly responsible for analgesic effects of oxycodone (Zwisler et al., 2009; Thompson et al., 2004). Conversely, several studies have suggested that the antinociceptive properties of oxycodone are mediated directly by the parent compound (Lalovic et al., 2006; Kaiko et al., 1996; Zwisler et al., 2010a; de Leon et al., 2003; Susce et al., 2006). Conclusive evidence of the contribution of CYP2D6 genotype in predicting efficacy and avoiding toxicity in breastfeeding mothers and infants has yet to be defined.
A recent study suggested that CYP2D6 and CYP3A are linked such that when the activity of one enzyme is reduced, the other enzyme compensates by increasing its metabolic rate (Samer et al., 2010a). CYP3A5 displays the highest activity for oxycodone N-demethylation in vitro (Lalovic et al., 2004). Like CYP2D6, CYP3A5 is polymorphic. Individuals homozygous for CYP3A5*3 have almost complete absence of the CYP3A5 protein resulting from alternative splicing and protein truncation. The CYP3A5*3 genotype shows ethnic differences in its frequency and is found in 77% of Japanese and up to 95% in Caucasians (Fukuen et al., 2002). Thus, this enzyme may be an important contributor to the interindividual differences in CYP3A-dependent clearance of oxycodone in vivo.

The highly polymorphic gene, OPRM1, encodes for the mu-opioid receptor (MOR1), the site of action for oxycodone. The allele frequency of the 118A>G single nucleotide polymorphism (SNP) in OPRM1 is 10-15% in the Caucasian population (Stamer and Stuber, 2007). Homozygous carriers of the mutant variant have reduced analgesic response to morphine (Campa et al., 2008; Reyes-Gibby et al., 2007) and oxycodone (Zwisler et al., 2010b) compared to homozygous or heterozygous wild type.

Since oxycodone’s site of action lies within the CNS, the efflux transporter P-glycoprotein (P-gp), encoded by the ATP-Binding Cassette B1 (ABCB1) gene, is an important determinant of its bioavailability in the brain (Wandel et al., 2002). The two most extensively studied SNP in this gene are 2677G>T/A and 3435C>T. In experimental pain studies, greater pain relief is achieved in homozygous variants compared to homozygous or heterozygous wildtype when oxycodone is administered (Zwisler et al., 2010b; Xie et al., 1999).

The aim of this study was to assess the effect of maternal CYP2D6, CYP3A5, ABCB1, and OPRM1 polymorphisms in predicting both neonatal and maternal CNS depression after oxycodone use during lactation.
3 Methods

3.1 Patient Recruitment

This was a nested case-control study (n=67) within a recently published larger cohort (n=139) (Lam et al., 2012a). Briefly, in our larger cohort study, a standardized follow-up questionnaire was administered during a telephone interview to assess adverse maternal and neonatal events, including CNS depression temporally related to oxycodone use during breastfeeding according to maternal self-reports compared to periods before and after oxycodone use. We also collected the number of consecutive hours the infants slept per day and if the infant required any medical interventions during the period of maternal oxycodone use. In addition, mothers were also asked to provide a saliva sample for genotyping. Infants identified with symptoms of sleepiness or lethargy during the period of drug exposure and the reversibility of CNS depression upon discontinuation of oxycodone use or breastfeeding as reported by the mother were classified as “symptomatic”. Furthermore, those mothers who reported experiencing sleepiness or lethargy during the period of oxycodone use were classified as “symptomatic”. Mothers who used oxycodone during the breastfeeding period, were ≥16 years of age, and provided informed consent to participate in the telephone interview were included in the study. On the other hand, women who did not provide consent to participate in the study, could not be contacted via telephone, did not breastfeed while using oxycodone, took other sedative medications beside oxycodone alone (such as other opioids, benzodiazepines, or skeletal muscle relaxants), used drugs of abuse or alcohol in late pregnancy or while breastfeeding, or whose infant was diagnosed with CNS anomalies were excluded from the study.

Only mothers who provided a saliva sample and provided consent for genotyping were included in our nested case-control study. They were sent written consent letters and Oragene® (DNA Genotek Inc., Kanata, ON, Canada) saliva collection cups labeled with barcode identification. Signed, written consent letters were returned to our office in pre-addressed envelopes, while barcode labeled saliva samples were sent back in prepaid packages to our
laboratory for DNA analysis. This study was approved by the Research Ethics Committee at the Hospital for Sick Children (Toronto, ON, Canada).

3.2 Genotyping

Evidence from clinical studies suggests that the CYP2D6, CYP3A5, ABCB1, and OPRM1 genes may be associated with the antinociceptive effects and adverse effects of oxycodone. Specific variants in these genes were selected for genotyping, including CYP2D6 (*3, *4, *5, *6, *7, *8, *9, *10, *12, *14, *17, *29, *41, *1xN, *2xN, *4xN, *41xN, CYP3A5*3, ABCB1 2677G>T/A and 3435C>T, and OPRM1 118A>G (Samer et al., 2010b; Volpe et al., 2011; Kaiko et al., 1996; Reyes-Gibby et al., 2007; Gronlund et al., 2010; Kummer et al., 2011; Gronlund et al., 2011; Heiskanen et al., 1998; Campa et al., 2008; Hassan et al., 2007; Naito et al., 2011; Bostrom et al., 2005). DNA was extracted from maternal saliva samples using an iPrep™ Purification Instrument and iPrep™ PureLink™ gDNA Blood Kit (Invitrogen, Burlington, ON, Canada) according to the manufacturer’s protocol. Polymorphisms in CYP2D6 were genotyped using a modification of a previously described protocol based on a multiplex single-base extension reaction (SNAPSHOT™; Applied Biosystems, Foster City, CA, USA) (Reyes-Gibby et al., 2007). The original protocol covering the variants *2, *3, *4, *6, *9, *10, *17, *29, *41 and whole gene deletion (*5) and duplications was extended to include additional non-functional variants *7, *8, *12, and *14 by adding respective detection primers to the multiplex SNAPSHOT reaction. Variants not carrying any detected mutations were classified as *1. CYP2D6 copy number was defined in whole-gene duplication positive cases with TaqMan® Copy Number Assay (Hs04502391_cn; Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol. Similarly, mutations in CYP3A5 (*3) and ABCB1 (rs2032582, rs1045642) were genotyped using TaqMan® Drug Metabolism Genotyping assays (C_26201809_30, C_11711720C_30, C_11711720D_40, C_7586657_20; Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s recommendations. A new SNAPSHOT multiplex assay was developed for the candidate polymorphism in OPRM1 (rs1799971). Briefly, the polymorphic genomic region was amplified using Platinum® Taq DNA Polymerase (Invitrogen, Burlington, ON, Canada), 10X PCR Buffer, MgCl₂, dNTPs, and primers, and amplified fragments were purified with ExoSAP-IT® (USB Products, Affymetrix, Inc., Cleveland, OH, USA), a
template in a SNaPshot reaction. Reaction conditions and temperature cycling were carried out according to manufacturer’s protocol. Using bidirectional Sanger sequencing, the genotyping results for each polymorphism in six samples representing different genotypes were confirmed. This suggested that the genetic results obtained from the various TaqMan® Assays were accurate.

3.3 Statistical Analysis

Statistical analysis was performed using SPSS (IBM SPSS, version 17, Somers, NY). Characteristics of mother and of neonates were compared by the Chi-square test or Fischer’s exact test for categorical variables and the Student’s t-test or one-way ANOVA were used for continuous variables. The maternal genetic polymorphisms of symptomatic infants were compared to those of asymptomatic infants with the Fischer’s exact test or Chi-square test when appropriate. A power calculation was performed to estimate the power of this study to detect the effect of CYP2D6 genotype on CNS depression.
4 Results

Sixty-seven women voluntarily provided saliva for genetic analysis out of a previously published cohort of 139 (Lam et al., 2012a). Forty-nine mothers refused genotyping and 23 mothers who initially accepted to be part of the genotype component of the study did not return their saliva to the lab for genetic analysis. The self-reported maternal indications for oxycodone use during breastfeeding were as follows: Cesarean delivery (n=25), vaginal delivery (n=1), headache/migraine (n=4), and dental/minor surgery (n=37). There were no differences between the maternal indication for oxycodone use during breastfeeding in symptomatic infants and asymptomatic infants (Table 3.1). There were no differences in maternal dose, ethnicity, parity, postmenstrual age (PMA) of infant at the time of oxycodone use, infant’s birth weight, frequency of breastfeeding and formula supplementation between mothers who did and did not report neonatal CNS depression (Table 3.1). None of the mothers were using drugs that inhibited CYP2D6, CYP3A4, and/or P-gp activity in this study. However, maternal age, and duration of breastfeeding (min/d) were significantly different between symptomatic and asymptomatic infants. Mothers of symptomatic infants used oxycodone for a longer period of time (number of days) during breastfeeding compared to those of asymptomatic infants (p<0.0001). Breastfeeding mothers of symptomatic infants were significantly more likely to experience CNS depression themselves after oxycodone use than those with asymptomatic infants (15/17 vs. 29/50, OR 5.43; 95% CI 1.00-38.57; P value=0.037).
Table 3.1 Demographic characteristics of oxycodone medicated mothers and their infants while breastfeeding

<table>
<thead>
<tr>
<th>Demographic characteristic, mean ± SD</th>
<th>Neonatal CNS Depression (n=17)</th>
<th>No Neonatal CNS Depression (n=50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>36.18± 3.07</td>
<td>32.56±4.52</td>
<td>0.033  a</td>
</tr>
<tr>
<td>Maternal dose (mg/kg/day)</td>
<td>0.24±0.19</td>
<td>0.20±0.14</td>
<td>0.66  a</td>
</tr>
<tr>
<td>Maternal non-Caucasian ethnicity (%)</td>
<td>3(18%)  *</td>
<td>6 (12%)  *</td>
<td>0.682  b</td>
</tr>
<tr>
<td>Parity (offspring)</td>
<td>1.76±1.15</td>
<td>1.62±0.92</td>
<td>0.730  a</td>
</tr>
<tr>
<td>Maternal sedation (%)</td>
<td>15 (88%)</td>
<td>29 (58%)</td>
<td>0.037  b</td>
</tr>
<tr>
<td>PMA of child (weeks)</td>
<td>45.33±9.64</td>
<td>48.48±11.59</td>
<td>0.15  a</td>
</tr>
<tr>
<td>Infant birth weight (kg)</td>
<td>3.49±0.63</td>
<td>3.24±0.50</td>
<td>0.101  a</td>
</tr>
<tr>
<td>Number of days of breastfeeding during oxycodone use (days)</td>
<td>6.71±1.53</td>
<td>2.92±1.63</td>
<td>&lt;0.0001 a</td>
</tr>
<tr>
<td>Duration of breastfeeding each day (minute/day)</td>
<td>46.18±53.58</td>
<td>151.64±99.23</td>
<td>&lt;0.001 a</td>
</tr>
<tr>
<td>Frequency of breastfeeding (times/day)</td>
<td>7.82±2.35</td>
<td>7.80±3.14</td>
<td>0.978 a</td>
</tr>
<tr>
<td>Formula supplementation (%)</td>
<td>7 (41%)</td>
<td>15 (30%)</td>
<td>0.55  b</td>
</tr>
</tbody>
</table>

Maternal Indications

<table>
<thead>
<tr>
<th>Maternal Indications</th>
<th>Neonatal CNS Depression (n=17)</th>
<th>No Neonatal CNS Depression (n=50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childbirth (Caesarian and vaginal delivery) (%)</td>
<td>4 (24%)</td>
<td>22 (44%)</td>
<td>0.160  b</td>
</tr>
<tr>
<td>Headache/migraine (%)</td>
<td>1 (6%)</td>
<td>3 (6%)</td>
<td>1.00  b</td>
</tr>
<tr>
<td>Dental/minor surgery (%)</td>
<td>12 (70%)</td>
<td>25 (50%)</td>
<td>0.168  b</td>
</tr>
</tbody>
</table>

a Student T-test, p< 0.05 denotes statistical significance

b Fischer Exact Test, p< 0.05 denotes statistical significance

* Grandparent ancestry: Guyana (1), Nigeria (2)

* Grandparent ancestry: Ghana (2), Canadian First Nation (1), Nigeria (1), India (1), Trinidad (1)
The *OPRM1 118A>G* variant (minor allele frequency (MAF) 10\%), *ABCB1 2677G>T/A* (MAF 42\%), *ABCB1 3435C>T* variant (MAF 43\%), and *CYP3A5*<sup>*</sup>3 (MAF 87\%) were in Hardy-Weinberg equilibrium. Genetic variants in the maternal *CYP2D6*, *ABCB1*, *CYP3A5*, and *OPRM1* genes alone did not correlate with oxycodone-induced CNS depression in infants (Table 3.2). One (5.9\%) of the mothers breastfeeding a symptomatic infant was a CYP2D6 UM compared to two (4.0\%) mothers breastfeeding asymptomatic infants [OR 1.5; 95\% CI 0.05-23.55; *P* value=1.0]. Genetic variants in *CYP2D6*, *CYP3A5*, and *OPRM1* were not significantly associated with oxycodone-induced maternal CNS depression (Table 3.3). However, mothers carrying at least one copy of the *ABCB1 2677 T* variant had an increased risk of experiencing sedation themselves [OR 2.35; 95\% CI 1.06-5.28; *P* value=0.03].
### Table 3.2 Maternal genetic variant significantly associated with oxycodone-induced CNS depression in infants.

<table>
<thead>
<tr>
<th>Genetic Variant</th>
<th>Predicted Activity</th>
<th>Symptomatic Infants (n=17)</th>
<th>Asymptomatic Infants (n=50)</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>**CYP2D6 genotype groups * **</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>CYP2D6 PM</em></td>
<td>Poor</td>
<td>1</td>
<td>5</td>
<td>1.00</td>
<td></td>
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<tr>
<td><em>CYP2D6 IM</em></td>
<td>Intermediate</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td><em>CYP2D6 EM</em></td>
<td>Extensive</td>
<td>14</td>
<td>43</td>
<td>1.14</td>
<td>(0.21-6.13)</td>
<td>1.000</td>
</tr>
<tr>
<td><em>CYP2D6 UM</em></td>
<td>Ultra-rapid</td>
<td>1</td>
<td>2</td>
<td>1.60</td>
<td>(0.14-18.91)</td>
<td>1.000</td>
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<tr>
<td>**OPRM1 rs1799971 (118A&gt;G) * **</td>
<td>Decreased</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>31</td>
<td>89</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>1</td>
<td>11</td>
<td>3.83</td>
<td>(0.48-82.60)</td>
<td>0.292</td>
</tr>
<tr>
<td>A/A</td>
<td></td>
<td>15</td>
<td>38</td>
<td>1.00</td>
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<tr>
<td>A/G</td>
<td></td>
<td>1</td>
<td>11</td>
<td>0.30</td>
<td>(0.03-2.61)</td>
<td>0.430</td>
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<tr>
<td>G/G</td>
<td></td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>**ABCB1 rs2032582 (2677G&gt;T/A) **</td>
<td>Decreased</td>
<td></td>
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<tr>
<td>G</td>
<td></td>
<td>22</td>
<td>57</td>
<td>1.00</td>
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<tr>
<td>A</td>
<td></td>
<td>0</td>
<td>1</td>
<td>NA</td>
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<tr>
<td>T</td>
<td>12</td>
<td>42</td>
<td>0.75</td>
<td>(0.31-1.81)</td>
<td>0.548</td>
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</tr>
<tr>
<td>G/G</td>
<td>7</td>
<td>19</td>
<td>1.00</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G/A</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>G/T</td>
<td>8</td>
<td>17</td>
<td>1.58</td>
<td>(0.52-4.81)</td>
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<tr>
<td>T/T</td>
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<td>13</td>
<td>0.38</td>
<td>(0.08-1.89)</td>
<td>0.320</td>
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</table>

**ABCB1 rs1045642 (3435C>T)**  Decreased

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<tbody>
<tr>
<td>C</td>
<td>20</td>
<td>54</td>
<td>1.0</td>
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<td></td>
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<tr>
<td>T</td>
<td>12</td>
<td>46</td>
<td>0.70</td>
<td>(0.29-1.71)</td>
<td>0.422</td>
</tr>
<tr>
<td>C/C</td>
<td>6</td>
<td>16</td>
<td>1.00</td>
<td></td>
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</tr>
<tr>
<td>C/T</td>
<td>8</td>
<td>22</td>
<td>1.27</td>
<td>(0.41-3.93)</td>
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</tr>
<tr>
<td>T/T</td>
<td>2</td>
<td>12</td>
<td>0.45</td>
<td>(0.09-2.28)</td>
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</tbody>
</table>

**CYP3A5 rs776746 (6986A>G)**  Decreased

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<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>10</td>
<td>1.00</td>
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<tr>
<td>G</td>
<td>21</td>
<td>90</td>
<td>0.33</td>
<td>(0.10-1.11)</td>
<td>0.056</td>
</tr>
<tr>
<td>A/A</td>
<td>2</td>
<td>1</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>3</td>
<td>8</td>
<td>1.43</td>
<td>(0.33-6.31)</td>
<td>0.693</td>
</tr>
<tr>
<td>G/G</td>
<td>9</td>
<td>41</td>
<td>0.40</td>
<td>(0.11-1.46)</td>
<td>0.270</td>
</tr>
</tbody>
</table>
NA, not available

* Specific SNP could not be genotyped for one case

§ Specific SNP could be not genotyped for three cases
Table 3.3 Genetic variants associated with oxycodone-induced CNS depression in mothers.

<table>
<thead>
<tr>
<th>Genetic Variant</th>
<th>Predicted Activity</th>
<th>Symptomatic Mothers (n=41)</th>
<th>Asymptomatic Mothers (n=26)</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2D6 genotype groups</strong> *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 PM</td>
<td>Poor</td>
<td>4</td>
<td>2</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 IM</td>
<td>Intermediate</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CYP2D6 EM</td>
<td>Extensive</td>
<td>35</td>
<td>22</td>
<td>1.27</td>
<td>(0.31-5.26)</td>
<td>0.730</td>
</tr>
<tr>
<td>CYP2D6 UM</td>
<td>Ultra-rapid</td>
<td>1</td>
<td>2</td>
<td>0.31</td>
<td>(0.03-3.58)</td>
<td>0.700</td>
</tr>
<tr>
<td><strong>OPRM1 rs1799971 (118A&gt;G)</strong> *</td>
<td>Decreased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>74</td>
<td>46</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>6</td>
<td>6</td>
<td>0.63</td>
<td>(0.17-2.35)</td>
<td>0.539</td>
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<tr>
<td>A/A</td>
<td></td>
<td>35</td>
<td>20</td>
<td>1.00</td>
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<td></td>
</tr>
<tr>
<td>A/G</td>
<td></td>
<td>4</td>
<td>6</td>
<td>0.37</td>
<td>(0.09-1.47)</td>
<td>0.175</td>
</tr>
<tr>
<td>G/G</td>
<td></td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>ABCB1 rs2032582 (2677G&gt;T/A)</strong></td>
<td>Decreased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>42</td>
<td>36</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>15</td>
<td>2.35 (1.06-5.28)</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>------------------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>13</td>
<td>13</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/T</td>
<td>16</td>
<td>9</td>
<td>1.21 (0.39-3.81)</td>
<td>0.800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>12</td>
<td>3</td>
<td>3.17 (0.70-16.19)</td>
<td>0.134</td>
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</tbody>
</table>

**ABCB1 rs1045642 (3435C>T)** * Decreased

<table>
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<tr>
<th></th>
<th>40</th>
<th>34</th>
<th>1.00</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>40</td>
<td>18</td>
<td>1.89 (0.87-4.14)</td>
</tr>
<tr>
<td>T</td>
<td>11</td>
<td>11</td>
<td>1.00</td>
</tr>
<tr>
<td>C/C</td>
<td>18</td>
<td>12</td>
<td>0.96 (0.32-2.89)</td>
</tr>
<tr>
<td>C/T</td>
<td>11</td>
<td>3</td>
<td>2.91 (0.64-15.02)</td>
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</table>

**CYP3A5 rs776746 (6986A>G)** § Decreased

<table>
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<tr>
<th></th>
<th>9</th>
<th>8</th>
<th>1.00</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>67</td>
<td>44</td>
<td>1.35 (0.43-4.20)</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>A/A</td>
<td>7</td>
<td>4</td>
<td>1.24 (0.28-5.85)</td>
</tr>
<tr>
<td>A/G</td>
<td>30</td>
<td>20</td>
<td>1.13 (0.29-4.34)</td>
</tr>
<tr>
<td>G/G</td>
<td>13</td>
<td>13</td>
<td>1.00</td>
</tr>
</tbody>
</table>
NA, not applicable

¶ Symptomatic mothers were defined as mothers who reported experiencing sleepiness or lethargy themselves during the period of oxycodone use

* Specific SNP could not be genotyped for one case

§ Specific SNP could not be genotyped for three cases
5 Discussion

This is the first nested case-control study to examine the contribution of maternal
CYP2D6, CYP3A5, ABCB1, and OPRM1 polymorphisms in predicting both maternal and neonatal
CNS depression. We found that mothers carrying at least one copy of the ABCB1 2677 T variant
had an increased risk of experiencing CNS depression. The exact mechanism of how the T
variant at position 2677 influences the transport activity of P-gp is not well understood.
Individuals genotyped as 3435TT in ABCB1 have 2-3 times lower P-gp expression than the wild-
type (Hoffmeyer et al., 2000). It is unknown at this time if the SNP 2677G>T/A alters expression
levels of P-gp, but researchers proposed that the variant allele of the SNP 2677G>T/A impairs
clearance of drugs out of the target cell (Sakurai et al., 2007). There is conflicting evidence
confirming the association between the ABCB1 SNP in 2677G>T/A and adverse drug reactions
of postoperative pain. Zwisler et al. showed that there was no significant difference in
drowsiness between wild-type and homozygous variants (Zwisler et al., 2010b). Wild type
individuals were found to have more severe nausea and vomiting, but carriers of the variant
allele have more headaches (Zwisler et al., 2010b). However, our result is consistent with the
impaired ability to clear oxycodone out of the brain for individuals carrying the variant allele in
the SNP 2677G>T/A leading to increased risk of experiencing CNS depression. A similar
association of the ABCB1 2677 T allele and maternal CNS depression was observed with
mothers breastfeeding on codeine (Sistonen et al., 2012).

None of the maternal genetic variants were associated with oxycodone-induced
depression in neonates or mothers. This may be attributed to the analgesic potency of the
parent drug, oxycodone. Unlike codeine, an inactive prodrug which relies on conversion into
morphine by CYP2D6, oxycodone is an active compound which illicits analgesic effects (Samer
et al., 2010b; Thompson et al., 2004; Kaiko et al., 1996). In this study, mothers of symptomatic
infants were equally as likely to be CYP2D6 UMs as those nursing asymptomatic infants. While
the parent compound may have been responsible for mediating oxycodone-induced CNS
depression in infants and mothers and not necessarily oxymorphone, the CYP2D6 UM mother
of the symptomatic infant was using a higher dose of oxycodone than the CYP2D6 UM mothers
of non-CNS-depressed infants (0.455mg/kg/day vs. 0.043mg/kg/day). Furthermore, the asymptomatic infants of CYP2D6 mothers had their feeding supplemented with formula since birth, lessening their exposure to oxycodone in breastmilk. Lastly, the CYP2D6 UM mother of the symptomatic case was using oxycodone for 7 days during breastfeeding when she noticed that the child presented signs of CNS depression, as opposed to 3 days in both asymptomatic infants. Thus, similar to our findings with codeine (Madadi et al., 2009), the interplay between clinical factors and maternal genotypes may protect the breastfed infant from sedative outcomes.

Lalovic et al. conducted a study to identify and assess the major cytochrome P450 enzymes responsible for the conversion of oxycodone to noroxycodone via N-demethylation using human liver microsomes (Lalovic et al., 2004). Although CYP3A represents the predominant oxidative pathway in human liver microsomes (>90%), several other P450s such as CYP2A6, 2C9 and 2C19 can mediate oxycodone N-demethylation but at a slower rate than CYP3A (Lalovic et al., 2004). Therefore, in those mothers carrying at least one copy of variant allele at CYP3A5*3 resulting in reduced CYP3A5 activity, other CYP pathways may compensate and metabolize oxycodone. This will prevent accumulation of the parent drug, reducing the risk of oxycodone-induced depression in the mother and her nursing infant. Future studies are needed to investigate the contribution of other CYPs or metabolic pathways such as glucuronidation to the metabolism of oxycodone.

Several authors have argued that although oxycodone and morphine are both opioid agonists at the MOR, they differ in terms of pharmacokinetics, pharmacodynamics, and genetics (Kalso, 2007). Although homozygous variants in the 118A>G SNP were associated with increased opioid requirements and less severe adverse effects, none of the studies included in a recent meta-analysis had patients using oxycodone (Walter and Lotsch, 2009). Thus it is not clear whether these findings for morphine are applicable for oxycodone.

Several important non-genetic factors previously described (Lam et al., 2012a) remained to be strong predictors of neonatal CNS depression. In this study, it appeared that breastfeeding on oxycodone for greater than 4 days was associated with CNS depression,
similar to the previous findings with codeine (Madadi et al., 2009). However, duration of breastfeeding (min/day) was significantly shorter in sedated infants, although they were exposed to oxycodone for a greater number of days. Maternal CNS depression remained a strong predictor of neonatal CNS depression as reported in the larger cohort (Lam et al., 2012a). This finding was also supported by an investigation by our group into the safety of codeine use during lactation (Madadi et al., 2009). However, 67% (44/67) of the mothers who used oxycodone during the postpartum period experienced CNS depression themselves. The incidence of maternal CNS depression associated with oxycodone exposure is approximately 3 times that of neonatal CNS depression. Furthermore, 66% (29/44) of the mothers who experienced CNS themselves were nursing non-CNS depressed infants.

Mothers of symptomatic infants were older than those of asymptomatic infants. Conflicting evidence exists about the effect of age on the incidence of side effects associated with opioid use. Several studies have shown that the duration of pain relief increases with age as older women achieve greater pain relief with the same dose of opioid compared to younger women (Kaiko, 1980; Bellville et al., 1971; VanderVaart et al., 2011). Yet another study suggests that the risk of CNS and respiratory depression increases significantly with age (Cepeda et al., 2003). Further statistical analysis revealed that symptomatic mothers were significantly older than those not experiencing sedation themselves (38.31±1.69 vs 29.58±2.84, OR 8.43 95% CI 7.63-9.83; p<0.0001) (Table S3.1). In addition, use of oxycodone greater than 4 days was also associated with maternal sedation while taking oxycodone.

This study has a number of limitations that need to be considered. First, our study had only a power of 9.21% to detect the effect of CYP2D6 genotype on neonatal CNS depression. Second, our sample did not carry some of the genotypes of interest. For example, none of our mothers were homozygous mutant for OPRM1 118G. Therefore, we were not able to determine if carrying two copies of the G allele in OPRM1 further protected these mothers from maternal CNS depression. Yet, the fact that among a large number of CNS-depressed mother-infant pairs these genotypes were not present suggests that their overall contribution may be minimal on a population-based level.
The ideal study to assess the contribution of maternal genotype in predicting toxicity in breastfeeding mothers and their infants when exposed to oxycodone would be prospectively designed. However, the clinical reality is that mothers and their infants are often released from the hospital shortly after birth, so ultimately it is left to the parents to observe for signs and symptoms of neonatal CNS depression in their breastfed babies and themselves. Therefore, reports of maternal and neonatal oxycodone-induced toxicity during the postpartum period are crucial to allow prescribers and regulatory agencies to detect, assess, understand, and prevent oxycodone-induced toxicities from occurring in the future (Lam et al., 2012b).

Our understanding of the contribution of genetic variations in drug-metabolizing enzymes and response to oxycodone has significantly advanced over the years. However, while the analgesic effect of oxycodone is less dependent on the formation of its CYP2D6 metabolite compared to codeine, the overall contribution of CYP2D6 genotype in predicting efficacy and avoiding toxicity in breastfeeding mothers is unknown. Thus, a larger sample is needed to assess the effect of maternal CYP2D6 and other genetic variants. In addition, we studied only 18 polymorphisms in 4 genes known to be involved in oxycodone metabolism and response. It is highly probable that other genetic variants may play a role in the variability observed with oxycodone metabolism and response. Thus, future studies are needed to investigate the contribution of other enzymes involved in the metabolism of oxycodone.

Future work is necessary to elucidate the mechanisms involved in infant susceptibility to oxycodone-induced toxicity. Since mothers who are prescribed oxycodone for the management of postpartum pain are often breastfeeding relatively young infants, it is important to investigate the contribution of ontogeny of CYP2D6 and relevant brain transporters in avoiding oxycodone-induced toxicity in the nursing infant. Recent studies on the ontogeny of CYP2D6 suggest that age and genetic determinants of CYP2D6 expression determine interindividual variability in CYP2D6-dependent metabolism. The ontogeny and genetic variants of relevant transporters in the brain leading to therapeutic failure or toxicity are unknown. Understanding how the interplay of developmental mechanisms, clinical and genetic factors contribute to neonatal oxycodone-induced toxicity when breastfed by mothers taking oxycodone will allow for the prescribing oxycodone to be safe for both the nursing mother and her baby.
In conclusion, our study suggests that prolonged maternal use of oxycodone for greater than 4 days increases the risk of CNS depression in the breastfed newborn. Over 65% of mothers given oxycodone in the postpartum period experience CNS depression, which is 17 times higher than mothers receiving codeine. Finally, mothers carrying at least one copy of the \textit{ABCB1 2677 T} variant are at an increased risk of experiencing CNS depression themselves.

6 Acknowledgment

This work was funded by a Post Market Drug Safety and Effectiveness Catalyst grant awarded by the Drug Safety and Effectiveness Network of the Canadian Institutes of Health Research (DSEN-CIHR). Gideon Koren is the recipient of the Research Leadership for Better Pharmacotherapy during Pregnancy and Breastfeeding (Hospital for Sick Children, Toronto, Canada) and the holder of Ivey Chair in Molecular Toxicology (Department of Medicine, University of Western Ontario). This work was presented at the 2012 meeting of the American Society for Clinical Pharmacology and Therapeutics (ASCPT; March 2012, Washington, DC). Jessica Lam was awarded the ASCPT Presidential Trainee award for this work.
7 Supplementary Table

(Not included in published article)

Table S3.1 Demographic characteristics of oxycodone-medicated symptomatic mothers and asymptomatic mothers.

<table>
<thead>
<tr>
<th>Demographic Characteristic, mean ± SD</th>
<th>Symptomatic Mothers (n=41)</th>
<th>Asymptomatic Mothers (n=26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>38.31±1.69</td>
<td>29.58±2.84</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maternal dose (mg/kg/day)</td>
<td>0.26±0.20</td>
<td>0.22±0.14</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parity (offspring)</td>
<td>1.83±1.04</td>
<td>1.62±1.32</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of days of oxycodone use (days)</td>
<td>6.93±1.24</td>
<td>2.47±1.42</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maternal non-Caucasian ethnicity (%)</td>
<td>5 (12%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4 (15%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Maternal Indications**

<table>
<thead>
<tr>
<th>Maternal Indications</th>
<th>Symptomatic Mothers (n=41)</th>
<th>Asymptomatic Mothers (n=26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childbirth (Caesarian and vaginal delivery) (%)</td>
<td>14 (34%)</td>
<td>12 (46%)</td>
<td>0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Headache/migraine (%)</td>
<td>1 (2%)</td>
<td>3 (12%)</td>
<td>0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dental/minor surgery (%)</td>
<td>26 (64%)</td>
<td>11 (42%)</td>
<td>0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Student T-test, <i>p</i> < 0.05 denotes statistical significance

<sup>b</sup> Fischer’s Exact Test, <i>p</i> < 0.05 denotes statistical significance

* Grandparent ancestry: Guyana (1), Nigeria (2), Trinidad (1)

* Grandparent ancestry: Ghana (2), Canadian First Nation (1), Nigeria (1)
8 References


Chapter 4 The Ontogeny of P-glycoprotein in the Developing Human Blood Brain Barrier: Implications for Opioid Toxicity in Neonates

This work is submitted for publication: Lam J, Baello S, Iqbal M, Kelly LE, Shannon PT, Chitayat D, Matthews SG, Koren G. The ontogeny of P-glycoprotein in the developing human blood brain barrier: Implications for opioid toxicity in neonates. Submitted.

[JL designed the study, collected the samples, collected the data, performed data analysis, and prepared the manuscript for submission].
1 Abstract

The objective of the study was to determine the ontogeny of P-gp in the human blood brain barrier (BBB). Postmortem cortex samples from gestational age (GA) 20-26 weeks, GA 36-40 weeks, postnatal age (PNA) 0-3 months, PNA 3-6 months, and adults were immunostained for P-gp. The intensity of P-gp staining in adults was significantly higher compared to at GA 20-26 weeks ($p=0.0002$), GA 36-40 weeks ($p=0.0002$), and PNA 0-3 months ($p=0.0044$). P-gp intensity at GA 20-26 weeks ($p=0.0011$), GA 36-40 weeks ($p=0.0013$), and PNA 0-3 months ($p=0.0173$) was significantly lower compared to at PNA 3-6 months. P-gp expression in the BBB is limited at birth, increases with postnatal maturation, and reaches adult levels at approximately 3-6 months of age. Given the immaturity of BBB P-gp after birth, morphine may concentrate in the brain. This provides mechanistic support to life threatening opioid toxicity seen with maternal codeine use during breastfeeding.

2 Introduction

P-glycoprotein (P-gp) is a membrane ATP binding cassette transporter present in many tissues such as the kidneys, liver, intestines, testes and brain (Thiebaut et al., 1987). Due to its strategic location on the luminal membrane of brain endothelial cells, it has been suggested that it plays an important role in limiting the entry of drugs into the brain (Schinkel et al., 1994; Schinkel, 1999). Morphine is commonly used for the management of mild to severe pain and different experimental approaches have demonstrated that P-gp influences the distribution of morphine across the blood brain barrier (BBB) (Dagenais et al., 2004; Zong and Pollack, 2000; Letrent et al., 1999; Thompson et al., 2000), and hence its analgesic, and adverse effects.

Neonates have been shown to have a heightened sensitivity to the central depressive effects of opioids compared to older infants and adults in both animal and human studies (Kupferberg and Leong Way, 1963; Bragg et al., 1995; Rai et al., 2005; Bouwmeester et al., 2003; Way et al., 1965; Koren et al., 1985). Furthermore, recent studies have documented that the newborn infant is sensitive to the respiratory effects of morphine when the mother is breastfeeding while taking codeine (Madadi et al., 2009; Lam et al., 2012). This may be
attributed to the limited expression of P-gp at the BBB during the neonatal period leading to an increased morphine concentration in the brain (Kupferberg and Leong Way, 1963; Bragg et al., 1995). P-gp has been detected as early as embryonic day 10.5 in the mouse brain and served as the earliest marker for endothelial cell differentiation during BBB development (Qin and Sato, 1995). P-gp gene expression in the mouse brain is low during late gestation and reaches adult levels by 3 weeks of life (Qin and Sato, 1995; Tsai et al., 2002; Ek et al., 2010).

In humans, P-gp immunoreactivity was detected as early as 8 to 12 weeks gestation in the fetal brain and its prevalence and intensity progressively increased with advancing age (Daood et al., 2008; Schumacher and Mollgard, 1997; Virgintino et al., 2008). However, even at term, P-gp expression in the fetus is low compared to adults (Daood et al., 2008). In contrast, the ontogeny of P-gp in the developing human infant has not been previously studied.

The objective of this study was to quantify the ontogeny of P-gp in endothelial cells of the developing human BBB. We hypothesized that P-gp expression would be low at birth and increase dramatically in the neonatal period, explaining why young infants are sensitive to the central effects of morphine during the first months of life compared to older infants and adults.

3 Materials and Methods

3.1 Subjects and Samples

Autopsies of adults and fetuses delivered at Mount Sinai Hospital in Toronto, Canada were reviewed to identify cases with no history of neurological pathology, no chromosomal anomalies, normal neurological examination and a formalin-fixed, paraffin-embedded postmortem cortex block available for study. Eight fetuses of 20 to 26 weeks gestation (GA 20-26 weeks) and eight fetus of age 36 to 40 weeks gestation (GA 36-40 weeks), representing the late second trimester and second half of the third trimester, were identified between 2010 and 2012. The fetal age was estimated on the basis of the crown-rump length and/or pregnancy records (last menstrual cycle and assessments of fetal physical maturity). In addition, eight adult subjects (mean age 53.6 ± 6.5 years old) were also identified between 2010 and 2012.
Autopsies of infants from the Department of Pathology at the London Health Science Center in London, Canada were reviewed to identify cases with no history of neurological pathology, no chromosomal anomalies, a normal neuropathological exam and a formalin fixed paraffin-embedded postmortem cortex block. Eight infants aged 0 to 3 months (PNA 0-3 months) and eight infants aged 3 to 6 months (PNA 3-6 months) were identified between 2009 and 2012. The infant age was estimated on the basis of the pregnancy records and neonatal physical maturity.

The data collected in this study were coded and analyzed anonymously. No personal identifiers were collected. Table 4.1 summarizes the characteristics and causes of death from the autopsy reports. This study was approved by the Research Ethics Board of the Mount Sinai Hospital in Toronto, Canada, University of Toronto in Toronto, Canada, University of Western Ontario in London, Canada and the London Health Science Center in London, Canada.

3.2 Immunohistochemistry

Coronal sections (5μm) were cut from the formalin-fixed, paraffin-embedded cortex blocks and mounted on positively charged microscopic slides. The samples were deparaffinized using xylene and rehydrated through a series of graded ethanol. The sections were rinsed (three times) with phosphate-buffered saline (PBS). The sections were incubated with 10nM sodium citrate with 0.05% Tween 20 (pH 6.0 at 97°C) for 30 min. The sections were incubated at room temperature in ammonium chloride (50nM; pH 7.4) with PBS for 10 min. The sections were blocked in 5% normal goat serum (1h; room temperature). Subsequently, the sections were incubated with the mouse monoclonal anti-P-gp antibody D-11 (diluted 1:50; Santa Cruz, CA) and double immunolabeled with rabbit anti-laminin antibody (diluted 1:50; Sigma-Aldrich) overnight at 4°C. After rinsing (5 times) with PBS, the sections were incubated (1h; room temperature) with Alexa Fluor 488 goat anti-mouse (diluted 1:1000; Life Technologies) and Alexa Fluor 594 chicken anti-rabbit (diluted 1:1000; Life Technologies) for P-gp and laminin detection, respectively. The sections were rinsed and incubated with 0.5μg/mL DAPI (Life Technologies; 10min; room temperature). Sections were rinsed with PBS and distilled water
before being incubated in copper sulfate (1mM; pH 5.0; 30min; room temperature). Sections were then rinsed with distilled water, PBS and mounted with DABCO.

3.3 Spinning Disc Confocal Microscopy and P-gp Quantification

Sections were viewed using the WaveFX Spinning Disc Confocal System by Quorum (Guelph, Ontario, Canada) with optimized Yokogawa CSU X1, Hamamatsu EM-CCD digital camera Image EM (C9100-13), and Leica DM16000B inverted research grade motorized microscope run by the Volocity 5.2.2 Acquisition software (Improvision, PerkinElmer, Massachusetts, USA). On the triple-immunolabeled section, a sequential scan procedure was applied during image acquisition of the three channels, Alexa Fluor 488 (excitation at 488nm and detection range 500-535nm; green fluorescence), Alexa Fluor 594 (excitation at 594nm and detection at 617nm; red fluorescence) and DAPI (excitation at 385-400nm and detection range 450-470nm; blue fluorescence). All images were acquired using constant imaging parameters for each channel [green channel: exposure time- 489ms, sensitivity-255, power 90%; red channel: exposure time- 689ms, sensitivity- 255, power- 90%, and blue channel: exposure time- 697ms, sensitivity- 255, power-90%]. Confocal images were taken at 1μm intervals through the z-axis of the section covering in total 6μm in depth. Images of 0.5mm x 0.5mm were recorded digitally and stored as TIFF files with the Volocity 5.2.2 Acquisition software (Improvision, PerkinElmer, Massachusetts, USA). Confocal imaging was performed at The Lunenfeld-Tanenbaum Research Institute in Mount Sinai Hospital, Toronto, Canada.

P-gp intensity was measured in seven brain microvessels from each section using Volocity Quantification 6.3 (Improvision, PerkinElmer, Massachusetts, USA). Brain microvessels for measurement were selected using the following criteria: 1) contained unbranched segments of at least 50μm in length, 2) appeared undamaged in transmitted light and fluorescence modes, and 3) the diameter of the microvessel was between 6-10μm. Two investigators (JL and SB) independently evaluated the selected microvessels. The selected microvessel was outlined manually using the region of interest tool to measure the average P-gp intensity of vessel. The fluorescence intensity was corrected for background.
3.4 Statistical Analysis

Statistical analysis was performed using SPSS (IBM, version 20, Somers, NY). The intensity of P-gp amongst the developmental age groups was compared using a one-way ANOVA followed by a Tukey’s multiple comparison test.

4 Results

P-gp immunoreactivity was detected as early as 20 weeks gestation and at all developmental ages. Fluorescence double labeling with laminin (red), a basement membrane protein, and P-gp (green) showed that P-gp in the cortex was exclusively localized to the brain microvessels at all developmental ages (Figure 4.1 A-T).
<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Gender</th>
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<tbody>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GA 20 weeks</td>
<td>F</td>
<td>Extreme prematurity</td>
</tr>
<tr>
<td>2</td>
<td>GA 21 weeks</td>
<td>M</td>
<td>IUD; premature rupture of membrane</td>
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<td>3</td>
<td>GA 23 weeks</td>
<td>M</td>
<td>Hypoplastic left heart syndrome</td>
</tr>
<tr>
<td>4</td>
<td>GA 25 weeks</td>
<td>F</td>
<td>IUD and placental insufficiency</td>
</tr>
<tr>
<td>5</td>
<td>GA 26 weeks</td>
<td>M</td>
<td>IUD and placental insufficiency</td>
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<tr>
<td>6</td>
<td>GA 36 weeks</td>
<td>M</td>
<td>IUD secondary to large retroplacental hemorrhage</td>
</tr>
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<td>7</td>
<td>GA 37 weeks</td>
<td>M</td>
<td>Abnormal cardiovascular morphology associated with severe tracheal stenosis and pulmonary maldevelopment</td>
</tr>
<tr>
<td>8</td>
<td>GA 38 weeks</td>
<td>M</td>
<td>IUD due to overcoiling of the umbilical cord and villous dysmaturity of placenta</td>
</tr>
<tr>
<td>9</td>
<td>GA 39 weeks</td>
<td>F</td>
<td>Acute chorioamnionitis with bronchopneumonia and epicardial abscesses</td>
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<td>10</td>
<td>GA 40 weeks</td>
<td>M</td>
<td>IUD and placental insufficiency</td>
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<td>11</td>
<td>GA 40 weeks</td>
<td>M</td>
<td>Overcoiling and placental dysmaturity associated with IUD</td>
</tr>
<tr>
<td>12</td>
<td>GA 40 weeks</td>
<td>M</td>
<td>Complex congenital heart disease</td>
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Infant

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<td>4.5 months</td>
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<td>5 months</td>
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<td>Metastatic synovial sarcoma with extensive tumor embolization to lungs</td>
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<td>Saddle embolus in pulmonary artery bifurcation and metastatic cholangiocarcinoma</td>
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<td>--------------</td>
<td>-----</td>
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<tr>
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<td>52 years old</td>
<td>M</td>
<td>Acute respiratory syndrome</td>
</tr>
<tr>
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<td>52 years old</td>
<td>M</td>
<td>Heavy acute bilateral bronchopneumonia</td>
</tr>
<tr>
<td>5</td>
<td>56 years old</td>
<td>M</td>
<td>Renal failure</td>
</tr>
<tr>
<td>6</td>
<td>57 years old</td>
<td>F</td>
<td>Ruptured sigmoid colon, right lower lobe pneumonia and rheumatic heart disease</td>
</tr>
<tr>
<td>7</td>
<td>59 years old</td>
<td>M</td>
<td>Dilated cardiomyopathy and coronary artery atherosclerosis</td>
</tr>
<tr>
<td>8</td>
<td>62 years old</td>
<td>F</td>
<td>Severe muscle atrophy, steatosis, bile stasis</td>
</tr>
</tbody>
</table>

M, Male; F, Female; IUD, Intrauterine death

*Fetal age estimated based on crown-rump length and/or pregnancy records (last menstrual cycle and assessments of fetal physical maturity)

*Infant age estimated based on pregnancy records and neonatal physical maturity
Figure 4.1 Human cortex tissue triple immunolabeled for P-gp, laminin, and DAPI at GA 21 weeks (A-D), GA 40 weeks (E-H), PNA 7 weeks (I-L), PNA 4 months (M-P), 57 year old adult (Q-T) and negative control (U).

Negative control confirmed the specificity of the antibody staining for P-gp (U). P-gp immunoreactivity was weak during the GA 21 and 40 weeks, moderate in PNA 7 weeks and strong at PNA 4 months and in the adult. P-gp immunoreactivity was exclusively localized to the brain microvessel in all sections regardless of age. All images taken at x100 original magnification.
The intensity of P-gp immunoreactivity progressively increased in the brain with increasing age (Figure 4.2). The intensity of P-gp in adults was significantly higher compared to that at GA 20-26 weeks ($p=0.0002$), GA 36-40 weeks ($p=0.0002$), and PNA 0-3 months ($p=0.0044$). Furthermore, there was less P-gp intensity at GA 20-26 weeks ($p=0.0011$), GA 36-40 weeks ($p=0.0013$), and PNA 0-3 months ($p=0.0173$) compared to at PNA 3-6 months. P-gp expression reaches adults levels by 3 to 6 months of age ($p=0.74$).

![Figure 4.2 Intensity of P-gp at various developmental ages.](image)

Seven brain microvessels from each sample were selected and the intensity of P-gp was quantified. The intensity of P-gp progressively increased during development, reaching adult levels by 3 to 6 months. Data expressed as mean ± SEM at each developmental age. P-gp intensity was compared using a one-way ANOVA followed by the Tukey’s multiple comparison test ($^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).
5 Discussion

P-gp expression at the BBB plays an essential role in limiting xenobiotic exposure through active efflux from the CNS. This is the first study examining the ontogeny of P-gp in the human BBB in the fetal, infant and adult period. Our results indicate that the P-gp immunostaining intensity was relatively low during the fetal and newborn period, but increased significantly with postnatal maturation. By PNA 3 to 6 months of life, P-gp intensity in microvessel endothelial cells of the cortex approximated that in adults. Thus the increased sensitivity to opioids in newborn infants compared to older infants may be attributable to the low expression of P-gp at birth and the resulting in increase in opioid transfer into the brain.

P-gp immunoreactivity was detected in microvessels of the cortex as early as GA 20 weeks suggesting that P-gp plays an important role in neuroprotection during fetal brain development. Previous studies in the human fetal brain detected P-gp in brain microvessels at crown-rump lengths corresponding to GA 17 to 24 weeks, serving as an early marker of BBB development (Schumacher and Mollgard, 1997; Virgintino et al., 2008; van Kalken et al., 1992). Virgintino and colleagues first detected P-gp as diffuse cytoplasmic labeling of the endothelial cells lining the cortical microvessels, which became linear and continuous on endothelial cell membranes at GA 22 weeks (Virgintino et al., 2008). Since Virgintino and colleagues did not investigate the expression of P-gp in the developing human cortex at GA 20 weeks, it is possible that P-gp may have already been expressed in the endothelial cells of the cortical microvessel before GA 22 weeks. Similarly, the youngest human fetal brain in the study, GA 22 weeks, stained positive for P-gp (Daood et al., 2008).

P-gp immunoreactivity in human cortex was confined to brain microvessels. Levels of P-gp increased with advancing age but did not reach adult levels by term indicating further postnatal changes. By 3 to 6 months of life, P-gp levels in the brain endothelial cells approximated those in adults. This developmental pattern mimics what has been reported in the mouse brain (Tsai et al., 2002). P-gp expression in the mouse brain was limited during late embryogenesis and the newborn period and increased remarkably with postnatal maturation reaching adult levels by day 21 of life (Tsai et al., 2002). In humans, the intensity of P-gp
immunostaining in the microvessel endothelial cells increased from GA 22 weeks up to term but the P-gp intensity at term was still significantly lower when compared to adults (Daoood et al., 2008).

Although the functional impact of normal developmental changes in BBB P-gp expression has not been thoroughly investigated, studies in adults have demonstrated that P-gp plays an active role in the barrier function by protecting the brain against the accumulation of toxic xenobiotics (Schinkel et al., 1994; Schinkel et al., 1995; Schinkel et al., 1996; Jette et al., 1995). Recently, it has been shown that developmental changes in BBB P-gp contribute to the accumulation of cyclosporine A, a P-gp substrate in the murine brain (Goralski et al., 2006). Specifically, cyclosporine A accumulation within the brain was the highest in newborn mice and decreased with increasing postnatal P-gp expression (Goralski et al., 2006). Hence, premature and full term neonates may be at risk for CNS drug toxicity due the limited BBB P-gp expression in the brain microvessels. Furthermore, neonates and young infants have been shown to be more sensitive to the central depressive effects of morphine compared to older children and adults in both animal and human studies (Kupferberg and Leong Way, 1963; Bragg et al., 1995; Rai et al., 2005; Bouwmeester et al., 2003; Way et al., 1965; Koren et al., 1985). Taken together with the findings from the present study, differences in BBB P-gp expression during brain development most likely enhance the CNS passage into the brain. As P-gp expression reaches adult levels by age 3 to 6 months, older infants have the capacity to efflux morphine from the brain before it has a chance to exert its depressant effect.

There are a number of limitations to the present study that should be acknowledged. Postmortem cortex samples were used in this study. P-gp immunostaining and intensity may be modulated by the conditions that proceed at the time of or after death such as hypoxia following intrauterine death, and umbilical cord or placental infarction. Intrauterine death comprises more than half of all fetal deaths (7/12). P-gp expression in murine brains was increased following hypoxia-induced treatment (Lazarowski et al., 2007). Thus hypoxia could have artificially increased the P-gp intensity in the fetal samples. P-gp immunostaining and intensity may also be modulated by the postmortem interval. However, the postmortem interval was unknown in this study. Only cortex samples were used in this study. Future studies
should investigate the ontogeny of P-gp in other brain regions and astrocytes. Lastly, genetic polymorphisms, which were not assessed in this cohort, have been shown to alter P-gp expression (Oh et al., 2005; Nakamura et al., 2002). Future studies should address the impact of genetic polymorphisms on P-gp mediated drug transport in newborns.

In conclusion, BBB P-gp expression is incomplete in the newborn period but increases rapidly after birth and reaches adult levels by 3 to 6 months. The limited BBB P-gp expression in newborns may allow drugs to concentrate in the brain, and as a consequence, lead to increased sensitivity to many drugs. Insight regarding the development of P-gp expression gained from this cohort furthers our understanding of the pharmacodynamics behind neonatal drug exposure.

6 Acknowledgement

The authors would like to thank Ryszard Bielecki for his excellent technical assistance with the spinning disc confocal microscope.
7 References


Chapter 5 Codeine-Related Deaths: The Role of Pharmacogenetics and Drug-Interactions


[JL collected the data, performed data analysis, and prepared the manuscript for submission. CJDR, MRH and BCC performed the genetic analysis].
1 Abstract

Objective: To assess the relationship between genetic polymorphisms and drug interactions on codeine and morphine concentrations in codeine-related deaths (CRD). Study Design: All CRD in Ontario, Canada between 2006 and 2008 were identified. Post-mortem blood was analyzed for 22 polymorphisms in 5 genes involved in codeine metabolism and response. Results: Sixty-eight CRD were included in this study. The morphine-to-codeine ratio was significantly correlated with the presence of a CYP2D6 inhibitor at varying potencies ($p=0.0011$). The presence of other central nervous system (CNS) depressants (i.e. benzodiazepines, hypnotics, and/or alcohol) was significantly associated with lower codeine concentrations as compared to CRD in which other CNS depressants were not detected ($p=0.0002$). Individuals who carried the $ABCB1 1236T$ variant had significantly lower morphine concentrations ($p=0.004$). Conclusion: In this population of individuals whose cause of death was related to codeine, drug interactions and genetic polymorphisms were significantly associated with post-mortem codeine and morphine concentrations.
2 Introduction

Over the last decade, the annual number of emergency department (ED) visits attributed to drug misuse or abuse has been steadily increasing. A report from the Drug Abuse Warning Network (DAWN) in 2011 estimated that over 1.2 million ED visits involved non-medical use of prescription medications in the United States. Twenty-nine percent of these visits were associated with non-medical use of prescription opioids (Drug Abuse Warning Network (DAWN), 2013). Overall, the number of medical emergencies involving non-medical use of opioids rose 183% from 2004 to 2011 in the United States (Drug Abuse Warning Network (DAWN), 2013). Coincident with the increased hospitalization involving prescribed opioids is a dramatic increase in the number of opioid-related deaths (Center for Disease Control and Prevention (CDC), 2012; Dhalla et al., 2009). The majority of these opioid-related deaths involved at least one non-opioid central nervous system (CNS) depressant (Dhalla et al., 2009). Concomitant use of opioids with benzodiazepines, hypnotics, and/or alcohol, may enhance the depressive effects of opioids on respiratory drive (Leander and Lucot, 1977; Haberman et al., 1995; Ruttenber et al., 1990).

Despite the known risks associated with concurrent use of opioids with non-opioid CNS depressants, it is difficult to document the toxicological effect of these drug interactions in human studies. In the absence of controlled-clinical trial data, toxicological data from post-mortem investigations are valuable, as they assist in the interpretation of drug concentrations in deaths involving opioid intoxication.

Codeine is a weak analgesic widely used in the management of mild-to-moderate pain. Morphine, the product of codeine O-demethylation by the highly polymorphic enzyme cytochrome P450 2D6 (CYP2D6), is the metabolite primarily responsible for the analgesic effect of codeine. The amount of morphine formed from codeine is highly variable, ranging from 0 to up to 75% (Gasche et al., 2004) of total codeine metabolism. Originally, CYP2D6 enzymatic activity was determined by the urinary ratio of a specific CYP2D6 substrate to its O-demethylated metabolite. Subsequently, genotyping methods have segregated the population into 4 phenotypes: poor metabolizer (PM), intermediate metabolizer (IM), extensive
metabolizer (EM), and ultra-rapid metabolizer (UM). It has been determined that PMs produce very limited morphine after codeine administration and experience inadequate pain relief (Desmeules et al., 1991; Thorn et al., 2009). On the other hand, UMs are at risk of experiencing opioid intoxication as a result of increased morphine production (Gasche et al., 2004; Koren et al., 2006). However, CYP2D6 genotype does not fully predict phenotype. In particular, concomitant use of a CYP2D6 inhibitor may mimic a PM phenotype, leading to discordance between genotype-to-phenotype predictions. In addition to CYP2D6, previous studies by our group and others suggest that polymorphisms in the UGT2B7, ABCB1, OPRM1, and COMT may be associated with both the anti-nociceptive and adverse effects of codeine and morphine (Gasche et al., 2004; Koren et al., 2006; Madadi et al., 2009; Eissing et al., 2012; Kelly et al., 2012; Sistonen et al., 2012).

The objective of the present study was to assess the relationship between genetic polymorphisms, drug interactions, and postmortem morphine and codeine concentrations in codeine-related deaths (CRD).

3 Material and Methods

3.1 Study Population

This study was approved by the Office of the Chief Coroner of Ontario (OCCO) and the Research Ethics Board of the Hospital for Sick Children in Toronto, Canada. In accordance with Ontario’s Coroners Act, all sudden and unexpected deaths, and/or unnatural deaths must be reported to the OCCO. Coroner’s death investigations involve classification of the medical cause of death. It also involves determination of the manner of death according to five categories: accident, homicide, natural, undetermined, and suicide. When necessary, a post-mortem examination that usually includes detailed toxicological testing is performed. Typical toxicological testing begins with screening for licit and illicit drugs by immunoassay and gas chromatography mass spectrometry (GC-MS), and screening for volatiles by headspace GC. This is followed by confirmation and quantitation by GC-MS or liquid chromatography (LC) - MS/MS, as required.
In a previous publication, population characteristics and descriptors associated with opioid-related fatalities in Ontario between 2006 and 2008 were described (Madadi et al., 2013). In this study, all drug-related deaths in which codeine was identified as a contributing factor to the fatality by the coroner were isolated. These deaths were attributed to codeine ingested alone or in combination with other pharmaceutical substances; deaths in which other circumstantial factors could have on their own resulted in fatality (i.e. homicide, external injuries, motor vehicle collisions, and disease) were not included. From this point, CRDs in which codeine, and its metabolite, morphine, were screened and quantified in femoral blood during the toxicological analysis were retrieved and reviewed. Using liquid chromatography tandem mass spectrometry, the limit of detection (LOD) for codeine and morphine was 32ng/mL and 15ng/mL, respectively. The limit of quantification (LOQ) for codeine and morphine was 63ng/mL and 32ng/mL, respectively. The data collected in this study were coded and analyzed anonymously. No personal identifiers were collected.

CRDs were excluded from the study if i) the use of heroin was suspected [based on detection of 6-monoacetylmorphine (6-MAM) in biological fluids, and/or the death scene investigation indicated that heroin may have been used], ii) morphine was suspected [based on the presence of morphine at the death scene investigation and/or a morphine prescription for the deceased], iii) there was an indication of codeine misuse (i.e., inappropriate route of codeine administration), iv) the manner of death was undetermined; v) femoral blood codeine and morphine concentrations were below the LOQ, and/or vi) samples were of insufficient quality or quantity for genotype analysis.

In this study, a drug interaction was defined as concurrent detection of codeine and a CYP2D6 inhibitor (Cozza et al., 2003; U.S Food and Drug Administration (FDA), 2011), or drugs known to act as a CNS depressant during the toxicological analysis. The detected CYP2D6 inhibitors were divided into 2 groups according to their inhibitory potency (Hemeryck and Belpaire, 2002). Strong inhibitors consisted of bupropion, fluoxetine, and paroxetine. Weak inhibitors consisted of citalopram, diphenhydramine, and sertraline. Furthermore, CNS depressants consisted of benzodiazepines (e.g., alprazolam, clobazam, clonazepam, diazepam,
lorazepam, oxazepam, and temazepam), hypnotics (e.g., zopiclone), ethyl alcohol, and/or other opioids (e.g., fentanyl, hydrocodone, hydromorphone, meperidine and oxycodone).

3.2 Genotyping

One milliliter of blood was collected from each CRD and used for genotype analysis. Genomic DNA was extracted from blood using the QiaSymphony DNA purification system (Qiagen, Toronto, Ontario, Canada) according to the manufacturer’s protocol. Polymorphisms in CYP2D6 were assessed using a modification of a previously described protocol based on the multiplex single-base extension reaction (SNAPshot™; Applied Biosystems, Foster City, CA, USA) (Sistonen et al., 2012) for variants *2, *3, *4, *6, *7, *8, *9, *10, *12, *14, *17, *29, *41 and whole gene deletion (*5) and duplications. Variants not carrying any detected mutations were classified as *1. CYP2D6 activity score assignments were based on the method described by Gaedigk et al. (Gaedigk et al., 2008).

Other genetic polymorphisms in the codeine and morphine pathway were also investigated. Morphine is a substrate of the efflux transporter P-glycoprotein (P-gp), which is encoded by the ABCB1 gene. Three variants in ABCB1 were targeted for genotyping (rs1128503, rs2032582, rs1045642). ABCB1 haplotypes using these three markers [C1236T, G2677T, and C3435T, respectively] have been shown to have an effect on the overall function of P-gp by means of conformational changes to the protein structure (Oh et al., 2005). The UDP-glucuronosyltransferase (UGT) 2B7 gene was also of interest as it largely mediates morphine glucuronidation into both active and inactive metabolites (Coffman et al., 1997). The UGT2B7*2 variant (rs62298861) is associated with increased UGT2B7 enzyme activity (Duguay et al., 2004). We also targeted the OPRM1 gene which encodes the mu opioid receptor. The OPRM1 118A>G variant (rs1799971) has been associated with reduced expression at the brain (Zhang et al., 2005). Lastly, we targeted the catechol O-methyltransferase gene, (COMT), which metabolizes catecholamines and has been associated with pain sensitivity (Lee et al., 2011). The COMT 389C>T, 611C>G/T, and 675G>A (rs4633, rs4818, rs4680, respectively) are suggested to contribute to pain perception and influence morphine-related side effects (Rakvag et al., 2005; Reyes-Gibby et al., 2007; Ahlers et al., 2013; Rakvag et al., 2008). Genotyping for ABCB1
(rs1128503, rs2032582, rs1045642), UGT2B7 (rs62298861), OPRM1 (rs1799971), and COMT (rs4633, rs4818, rs4680) were all conducted using TaqMan® Genotyping assays (Life Technologies/Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s recommendations as described previously (Sistonen et al., 2012). Genotyping for the UGT2B7*2 variant was conducted by RFLP as described previously (Sistonen et al., 2012).

3.3 Statistical Analysis

Statistical analysis was performed using SPSS (IBM, version 20, Somers, NY). Characteristics of CRDs in which the manner of death was deemed to be accidental or suicide were compared by the Chi-square test or Fischer’s exact test for categorical variables and Student’s t-test and one-way ANOVA for continuous variables. The M/C ratio, codeine concentration, and morphine concentration among the genetic polymorphisms of interest were analyzed using the Mann-Whitney U and Kruskal-Wallis test when appropriate. Multivariate linear regression models were constructed to identify independent factors associated with post-mortem M/C ratio, codeine concentrations and morphine concentrations. For all three regression models, the independent variables that were included were manner of death, CYP2D6 genotype, presence of CYP2D6 inhibitor, and presence of additional CNS depressant.
4 Results

In the study period, there were 1359 deaths that were attributed to opioids in Ontario (Madadi et al., 2013). Among these, codeine was detected during toxicological analysis in 208 cases. Of these, 68 CRDs were included in this study (Figure 5.1). Cases were excluded for the following reasons: deaths were unrelated to codeine as deemed by the coroner (n=1151), codeine and/or morphine concentration was less than the LOQ (n=41), morphine was not screened (n=22), manner of death was deemed undetermined by the coroner (n=27), heroin or morphine use was suspected (n=5), drug injection or inhalation was identified (n=16), and/or sufficient blood was not available for genotyping (n=29).

![Diagram showing the overview of inclusion and exclusion criteria for CRD in this study.]

Figure 5.1 Overview of inclusion and exclusion criteria for CRD in this study.
Individuals whose manner of death was suicide were more likely to have a weak CYP2D6 inhibitor detected in the toxicological screen \( (p=0.05) \) than those in which the manner of death was accidental (Table 5.1). Furthermore, suicides were associated with higher codeine concentration than accidental deaths (median 455 ng/mL (inter-quartile range (IQR) 252.5-1250) vs. 315 ng/mL (IQR: 192.5-522.5), \( p=0.038 \)) (Figure 5.2). For cases in which other CNS depressants were detected, codeine and morphine concentrations were lower than those cases in which no concomitant CNS depressants were detected (median 320 ng/mL (IQR: 172.5-545) vs. 1200 ng/mL (IQR: 450-2000), \( p=0.0002 \)) (Figure 5.3).
Table 5.1 Comparison of demographic, health and death characteristics by the manner of death whose cause of death was related to codeine in Ontario for years 2006, 2007, and 2008.

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<td>50 (42-56)</td>
<td>0.878(^1)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>18 (50%)</td>
<td>18 (56%)</td>
<td>0.635</td>
</tr>
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<td>Health and Disease Characteristics (%)</td>
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<td></td>
</tr>
<tr>
<td>Bipolar/Schizophrenia</td>
<td>4 (11%)</td>
<td>4 (13%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Depression</td>
<td>3 (8%)</td>
<td>12 (38%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>0.471</td>
</tr>
<tr>
<td>History of Alcohol Abuse</td>
<td>3 (8%)</td>
<td>1 (3%)</td>
<td>0.616</td>
</tr>
<tr>
<td>History of Illegal Drug Abuse</td>
<td>3 (8%)</td>
<td>0 (0%)</td>
<td>0.241</td>
</tr>
<tr>
<td>Characteristics of Death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other opioids detected on toxicological screen besides codeine and its metabolite, morphine (%)</td>
<td>16 (44%)</td>
<td>12 (38%)</td>
<td>0.626</td>
</tr>
<tr>
<td>Ethanol Detected (%) (^2)</td>
<td>11 (31%)</td>
<td>12 (38%)</td>
<td>0.613</td>
</tr>
<tr>
<td>Mean ± SD ethanol concentration</td>
<td>210 ± 124 mg/100mL</td>
<td>123 ± 91 mg/100mL</td>
<td>0.080 (^3)</td>
</tr>
<tr>
<td>Illegal Drugs Detected (%)</td>
<td>7 (18%)</td>
<td>3 (9%)</td>
<td>0.326</td>
</tr>
<tr>
<td>CYP2D6 Inhibitor Detected (%)(^4)</td>
<td>16 (44%)</td>
<td>19 (59%)</td>
<td>0.236</td>
</tr>
<tr>
<td>Strong CYP2D6 Inhibitor (^5) Detected (%)</td>
<td>7 (19%)</td>
<td>3 (9%)</td>
<td>0.314</td>
</tr>
<tr>
<td>Weak CYP2D6 Inhibitor (^6) Detected (%)</td>
<td>11 (31%)</td>
<td>18 (56%)</td>
<td>0.05</td>
</tr>
<tr>
<td>CYP3A4 Inhibitor (^7) Detected (%)</td>
<td>2 (5%)</td>
<td>1 (3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>CNS Depressant Detected (%)(^8)</td>
<td>28 (78%)</td>
<td>25 (78%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Metabolic Ratio (Morphine-to-Codeine) (median (IQR))</td>
<td>0.0045 (0-0.0958)</td>
<td>0.0013 (0-0.0945)</td>
<td>0.679 (^1)</td>
</tr>
</tbody>
</table>
All tests were performed by Chi-square unless otherwise indicated

1 Mann-Whitney U-test

2 Ethanol detected in femoral blood

3 Student’s t-test

4 Case may have both a weak and strong CYP2D6 inhibitor detected during the toxicological analysis

5 Strong CYP2D6 inhibitor: bupropion, fluoxetine, paroxetine

6 Weak CYP2D6 inhibitor: citalopram, diphenhydramine, sertraline

7 CYP3A4 inhibitor: diltiazem

8 Detection of other opioids, benzodiazepines, hypnotics, alcohol, and/or tricyclic antidepressants during the toxicological analysis
Figure 5.2 Distribution of femoral blood concentration (ng/mL) per manner of death.
Higher codeine concentration quantified in the toxicological analysis in deaths deemed suicidal compared to those deemed accidental ($p=0.038$). The median and interquartile ranges (IQR) were depicted in the graph for each group.
Figure 5.3 Distribution of femoral blood codeine concentration (ng/mL) in 68 CRD cases where no additional CNS depressants (n=11) and additional CNS depressants (n=57) were detected during toxicological analysis.

Higher concentrations quantified in the toxicological analysis in deaths where no additional CNS depressants were detected compared to those where addition CNS depressants were detected ($p=0.0002$). The median and interquartile ranges (IQR) were depicted in the graph for each group.

There was a significant decrease in the M/C ratio when CYP2D6 inhibitors were detected (n=41) as compared to when they were not detected (n=27) within the whole cohort of 68 decedents (median 0 (IQR: 0-0.07) vs. 0.0467 (IQR: 0-0.12), $p=0.05$). We divided the CYP2D6 inhibitors into 2 groups according to their inhibitory potency (Hemeryck and Belpaire, 2002). Strong inhibitors consisted of bupropion, fluoxetine, and paroxetine (n=11). Weak inhibitors consisted of citalopram, diphenhydramine, and sertraline (n=29). The M/C ratio was
statistically different in the absence of a CYP2D6 inhibitor, in the presence of a weak CYP2D6 inhibitor, in the presence of a strong inhibitor, and in individuals genotyped as a CYP2D6 PM ($p=0.0011$) (Figure 5.4). There was a more pronounced decrease in M/C ratio with strong CYP2D6 inhibitors compared to weak CYP2D6 inhibitors and the absence of any CYP2D6 inhibitors [median 0 (IQR: 0-0.02) vs. 0.05 (IQR: 0-0.22), $p=0.0015$ and median 0 (IQR: 0-0.02) vs. 0.05 (IQR: 0-12), $p=0.0012$, respectively]. Furthermore, the M/C ratio in CYP2D6 PMs was significantly lower than those with a weak CYP2D6 inhibitor and the absence of a CYP2D6 inhibitor (median 0 (IQR: 0-0.008) vs. 0.05 (IQR: 0-0.22), $p=0.03$ and median 0 (IQR: 0-0.008) vs. 0.05 (IQR: 0-0.12), $p=0.03$, respectively).

![Figure 5.4](image-url)

**Figure 5.4** Effect of concurrent detection of CYP2D6 inhibitor during toxicological analysis on morphine-to-codeine ratio in CRD.

The morphine-to-codeine ratio significantly decreased with increased potency of CYP2D6 inhibitor with CYP2D6 PM having the lowest morphine-to-codeine ratio ($p=0.0011$). The median and interquartile ranges (IQR) were depicted in the graph for each group.
CYP2D6 genotype was not significantly associated with M/C ratio ($p=0.20$, Table 5.2). However, with increasing CYP2D6 activity scores (Gaedigk et al., 2008), there was a trend towards increased M/C ($p=0.87$, Table 5.2). None of the other variants in *UGT2B7, COMT*, or *OPRM1* were significantly associated with M/C ratio, morphine concentration, or codeine concentration (Table 5.2). However, individuals who carried the *ABCB1 1236 T* variant had statistically lower morphine concentrations than wild-type carriers (median 0 ng/mL (IQR: 0-46) vs. 17 ng/mL (IQR: 0-360), $p=0.004$) (Figure 5.5). After multivariate adjustment, none of the independent variables predicted the M/C ratio, femoral codeine and morphine blood concentrations.
Table 5.2 Postmortem femoral blood M/C ratio, codeine concentrations and morphine concentrations in CRD in this study (median (IQR)).

<table>
<thead>
<tr>
<th>Genetic Variant</th>
<th>Predicted Activity</th>
<th>Frequency (%)</th>
<th>Codeine Concentration (ng/mL)</th>
<th>P value</th>
<th>Morphine Concentration (ng/mL)</th>
<th>P value</th>
<th>M/C ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td><strong>CYP2D6 Genotype Groups</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6 PM</strong></td>
<td>Poor</td>
<td>8/68 (12%)</td>
<td>330 (172.5-425)</td>
<td>0.22</td>
<td>0 (0-21)</td>
<td>0.36</td>
<td>0 (0-0.01)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>CYP2D6 IM</strong></td>
<td>Intermediate</td>
<td>23/68 (34%)</td>
<td>445 (252.2-1450)</td>
<td>1.5</td>
<td>0 (0-94.3)</td>
<td>0</td>
<td>0 (0-0.64)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6 EM</strong></td>
<td>Extensive</td>
<td>30/68 (44%)</td>
<td>400 (160-170)</td>
<td>26</td>
<td>0 (0-150)</td>
<td>0.03</td>
<td>0 (0-0.22)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6 UM</strong></td>
<td>Ultra-rapid</td>
<td>7/68 (10%)</td>
<td>370 (180-530)</td>
<td>17</td>
<td>0 (0-100)</td>
<td>0.08</td>
<td>0 (0-0.12)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6 Activity Scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No activity</td>
<td>8/68 (12%)</td>
<td>330 (172.5-425)</td>
<td>0.79</td>
<td>0 (0-21)</td>
<td>0.69</td>
<td>0 (0-01)</td>
<td>0.87</td>
</tr>
<tr>
<td>0.5</td>
<td>Moderate activity</td>
<td>0/68 (0%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Moderate activity</td>
<td>10/68 (15%)</td>
<td>395 (240-860)</td>
<td>0.5</td>
<td>0 (0-60)</td>
<td>0.02</td>
<td>0 (0-0.04)</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>Moderate activity</td>
<td>13/68 (19%)</td>
<td>460 (295-650)</td>
<td>2.7</td>
<td>0 (0-94.3)</td>
<td>0.028</td>
<td>0 (0-0.08)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal activity</td>
<td>30/68 (44%)</td>
<td>400 (160-170)</td>
<td>26</td>
<td>0 (0-150)</td>
<td>0.03</td>
<td>0 (0-0.22)</td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>High activity</td>
<td>7/68 (10%)</td>
<td>370 (180-530)</td>
<td>17</td>
<td>0 (0-100)</td>
<td>0.08</td>
<td>0 (0-0.12)</td>
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</tr>
<tr>
<td><strong>UGT2B7 rs62298861 (A&gt;G)</strong> 2</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>Normal</td>
<td>18/67 (27%)</td>
<td>510 (227.5-1375)</td>
<td>0.08</td>
<td>1.5 (0-77.8)</td>
<td>0.73</td>
<td>0.01 (0-0.09)</td>
<td>0.58</td>
</tr>
<tr>
<td>Genotype</td>
<td>Genotypic Frequency</td>
<td>Genotypic Frequency (×100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>----------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*2</td>
<td>30/67 (45%)</td>
<td>430 (265-580)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0 (0-53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0-0.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>*2/*2</td>
<td>Increased</td>
<td>19/67 (28%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>220 (160-402.5)</td>
<td>23.5 (0-115)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.007 (0-0.2)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**OPRM1 rs1799971 (118A>G)**  
Decreased

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotypic Frequency</th>
<th>Genotypic Frequency (×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>50/68 (74%)</td>
<td>390 (217.5-912.5)</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>17 (0-150)</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>0 (0-0.12)</td>
</tr>
<tr>
<td>A/G</td>
<td>18/68 (26%)</td>
<td>400 (157.5-670)</td>
</tr>
<tr>
<td></td>
<td>0 (0-29)</td>
<td>0.0048 (0-0.044)</td>
</tr>
</tbody>
</table>

**ABCB1 rs1128503 (1236C>T)**  
Decreased

<table>
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<th>Genotypic Frequency</th>
<th>Genotypic Frequency (×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>29/68 (43%)</td>
<td>420 (230-1100)</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>17 (0-360)</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0 (0-0.12)</td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>27/68 (40%)</td>
<td>360 (192.5-535)</td>
</tr>
<tr>
<td></td>
<td>14 (0-51.75)</td>
<td>0.007 (0-0.10)</td>
</tr>
<tr>
<td>T/T</td>
<td>12/68 (18%)</td>
<td>190 (130-530)</td>
</tr>
<tr>
<td></td>
<td>0 (0-15)</td>
<td>0 (0-0.038)</td>
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</table>

**ABCB1 rs2032582 (2677G>T/A)**  
Decreased

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<th>Genotypic Frequency</th>
<th>Genotypic Frequency (×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>22/68 (32%)</td>
<td>400 (170-1100)</td>
</tr>
<tr>
<td></td>
<td>0.58</td>
<td>17 (0-194)</td>
</tr>
<tr>
<td></td>
<td>0.79</td>
<td>0.02 (0-0.14)</td>
</tr>
<tr>
<td></td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>GT/A</td>
<td>35/68 (51%)</td>
<td>395 (240-590)</td>
</tr>
<tr>
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<td>0 (0-53.8)</td>
<td>0 (0-0.09)</td>
</tr>
<tr>
<td>TT/TA/AA</td>
<td>11/68 (17%)</td>
<td>370 (135-705)</td>
</tr>
<tr>
<td></td>
<td>0 (0-120)</td>
<td>0.004 (0-0.05)</td>
</tr>
</tbody>
</table>

**ABCB1 rs1045642 (3435C>T)**  
Decreased

<table>
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<th>Genotype</th>
<th>Genotypic Frequency</th>
<th>Genotypic Frequency (×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>22/68 (32%)</td>
<td>410 (232.5-1150)</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>22.5 (0-196.3)</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>0.023 (0-0.21)</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>33/68 (49%)</td>
<td>370 (195-555)</td>
</tr>
<tr>
<td></td>
<td>3.9 (0-53.5)</td>
<td>0.001 (0-0.09)</td>
</tr>
<tr>
<td>T/T</td>
<td>13/68 (19%)</td>
<td>320 (135-705)</td>
</tr>
<tr>
<td></td>
<td>0 (0-150)</td>
<td>0 (0-0.03)</td>
</tr>
</tbody>
</table>

**ABCB1 Haplotype (1236-2677-3435)**

<table>
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<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Genotypic Frequency (×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGC</td>
<td>High</td>
<td>17/68 (25%)</td>
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<tr>
<td></td>
<td></td>
<td>320 (200-560)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 (0-80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 (0-0.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52</td>
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</tr>
<tr>
<td>TTT</td>
<td>Decreased</td>
<td>41/68 (61%)</td>
</tr>
<tr>
<td>CGT</td>
<td>Decreased</td>
<td>5/68 (7%)</td>
</tr>
<tr>
<td>&lt;5.0% frequency</td>
<td></td>
<td>5/68 (7%)</td>
</tr>
</tbody>
</table>

**COMT Haplotype rs4633-rs4818-rs 4680 (389C>T-611C>G-675G>A)**

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>CGG</td>
<td>High</td>
<td>31/68 (46%)</td>
<td>390 (150-460)</td>
<td>0.47</td>
<td>34 (0-80)</td>
<td>0.39</td>
</tr>
<tr>
<td>TCA</td>
<td>Decreased</td>
<td>32/68 (47%)</td>
<td>420 (200-635)</td>
<td>15 (0-100)</td>
<td>0.02 (0-0.47)</td>
<td></td>
</tr>
<tr>
<td>CCG</td>
<td>Very low</td>
<td>5/68 (7%)</td>
<td>320 (167-470)</td>
<td>0 (0-19)</td>
<td>0 (0-0.56)</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available

1 All tests were performed by Kruskal-Wallis test, $p<0.05$ denotes statistical significance

2 Specific SNP could not be genotypes for one case.
Figure 5.5 Effect of *ABCB1* 1236C>T genotype on femoral blood morphine concentration (ng/mL).

Individuals with one or both variant alleles had lower morphine concentrations than those genotyped to be *ABCB1* 1236CC (*p* = 0.040). The median and interquartile ranges (IQR) were depicted in the graph for each group.

5 Discussion

This study provides the first analysis of the relationship between genotype and drug interactions on postmortem codeine and morphine concentrations in CRDs. The results indicate that the strength of a CYP2D6 inhibitor used concomitantly with codeine is significantly correlated with M/C ratios observed in post-mortem samples. In this study, approximately 51% of the deaths involved the use of a CYP2D6 inhibitor, commonly an antidepressant, concurrently with codeine. Furthermore, the concomitant use of a weak or strong CYP2D6 inhibitor was associated with a significant decrease in M/C ratio. Similarly, experimental studies in living patients have shown that CYP2D6 inhibition (with fluoxetine or quinidine) may
cause individuals with a CYP2D6 EM genotype to exhibit a functional PM status (Fernandes et al., 2002; Sindrup et al., 1996).

The co-prescription of opioids with CYP2D6 inhibitors and/or other CNS depressants appears to be common in chronic pain populations (Pergolizzi et al., 2011). Our current study illustrates that approximately 84% (57/68) of CRD involved either an additional CNS depressant or CYP2D6 inhibitor and that 88% of these multidrug deaths involving codeine were deemed accidental. These results also suggest that codeine, when used in combination with other centrally acting drugs, was more toxic than codeine used alone; as evidenced by lower codeine femoral blood concentrations in multi CNS-drug deaths compared to single codeine-related deaths. Enhanced toxicity of alcohol and other centrally acting drugs with opioids is evident in animal studies (Leander and Lucot, 1977; Rattan and Sribanditmongkol, 1994; Garty et al., 1985) and in reports of opioid overdose fatalities (Puschel, 1993; Darke et al., 1997; Gossop et al., 2002). Consistent with our findings with codeine, Cone and colleagues found that in deaths involving oxycodone in combination with other centrally acting drugs, mean oxycodone blood concentrations were lower as compared to oxycodone-only deaths (Cone et al., 2004). The mechanism by which the combination of opioids with other CNS depressants significantly increases the risk of mortality is not completely understood but is thought to occur by means of additive or synergistic effects on the respiratory center. Our results warrant a further examination on the safety of concomitant prescriptions for CNS depressants with opioids.

Although we did not find an association between CYP2D6 genotype and M/C ratio, there was a trend between increasing CYP2D6 activity and M/C ratio. The presence of a CYP2D6 inhibitor and the manner of death may have played a role. A large proportion of codeine-related fatalities in this study had a CYP2D6 inhibitor detected on the toxicological analysis, which may have affected the production of morphine regardless of CYP2D6 genotype. Furthermore, we found that suicide cases had higher codeine concentration than accidental deaths. Acute ingestion of large quantities of codeine in suicidal cases may have saturated the capacity of CYP2D6, as has been demonstrated with nortriptyline (Vandel et al., 1990; Jerling and Alvan, 1994). Also, there may not have been sufficient amount of time for the biotransformation of codeine into morphine to occur in suicides in which a large amount of
codeine was taken at once. Finally, the measured codeine and morphine concentrations are potentially subject to post-mortem changes such as post-mortem drug redistribution. There is limited data indicating that codeine and morphine may exhibit post-mortem redistribution, but the results are equivocal and subject to several variables, including postmortem interval (Gerostamoulos and Drummer, 2000; Tolliver et al., 2010; Logan and Smirnow, 1996; Crandall et al., 2006). The postmortem interval could not be reliably estimated in this study. The time between when the death was pronounced and the autopsy/sample collection was performed ranged between 2 to 72 hours in this study. However, individuals may have been dead for variable durations before the body was discovered. Therefore, all these factors may complicate the relationship between the CYP2D6 genotype and observed postmortem M/C ratio.

We found that individuals who carried one or both variant alleles in the ABCB1 1236 C>T gene had lower morphine concentrations than those who were homozygous wild-types. The efflux transporter, P-gp is encoded by the ABCB1 gene and is a major determinant of the intracellular concentration of morphine within the brain (Somogyi et al., 2007). ABCB1 haplotypes that include 1236C>T have been shown to alter the function and protein structure of P-gp (Oh et al., 2005). It has been postulated that these variants may reduce the expression of P-gp at the luminal membrane of the brain endothelial cells, thereby rendering individuals as more sensitive to the depressive effects of morphine due to higher CNS morphine concentrations (Bourasset and Scherrmann, 2006; Xie et al., 1999). The specific impact of the ABCB1 1236C>T on P-gp activity is not known and needs further study.

This study has a number of limitations. Our analysis was based on a single postmortem codeine and/or morphine concentration and could not capture ante-mortem parameters such as codeine dose ingested, acute and/or chronic codeine use, and timing of codeine administration prior to fatality. We also did not have information on the ethnicity of the deceased; different ancestries may have basal differences in CYP2D6 activity. Furthermore, variable circumstances surrounding each CRD may have led to differences in the way the death investigation was conducted (i.e., amount of sample available for analysis, death scene circumstances, the availability of witness and/or family information). It is also possible that
toxicological testing may have not identified additional drugs (including other CYP2D6 inhibitors) that could have affected the results.

Our findings demonstrate that the interplay of several factors such as genetic polymorphisms, concomitant CNS depressants, and drug interactions may affect codeine and morphine concentrations detected postmortem, and ultimately the M/C ratio. These factors should be considered as part of a comprehensive interpretation of postmortem codeine and/or morphine concentrations. Given the high observed incidence of multidrug toxicity amongst codeine-related deaths, the safety of prescribing concomitant CNS depressants and/or CYP2D6 inhibitors together with codeine (and similar opioids) should be further studied.

6 Acknowledgement

The authors would like to thank the Office of the Chief Coroner of Ontario for their support of this research.
7 References


Chapter 6 Summary of Research Findings and Discussion

The use for opioid analgesia for the management of pain has increased over the last decade. A wealth of studies warns of the dangers of using opioid analgesics especially in susceptible populations such as mothers and their infants (Koren et al., 2006; Madadi et al., 2010; Timm, 2013). Although opioids are effective in relieving pain for many, treatment outcomes and adverse events are highly variable among patients. Currently, there is no well-validated manner of identifying those patients who will experience adequate analgesia at standard doses with minimal side effects. Personalized medicine, optimizing the medication type and dose for individual patients based on genetic biomarkers and other patient-related factors is not a new concept (Bruehl et al., 2013). Its application to the field of pain management is promising but is still too early to be implemented into clinical practice. A critical question that remains to be answered is what are the predictors of opioid toxicity? Specifically, before initiating opioid therapy, how do genotypic and phenotypic factors serve as predictors of opioid response and toxicity?

The present chapter discusses the significance of the specific research findings outlined in the preceding chapters, and summarizes these findings in terms of how they can be used as predictors for opioid-induced toxicity and aid in increasing both the effectiveness and safety of opioids in the clinical setting.

1 Summary and Significance of Research Findings

1. Determining whether the replacement of codeine by oxycodone is safe for both the mother and infant by quantifying the incidence of CNS depression in neonates reported by mothers taking oxycodone during breastfeeding compared to infants breastfed by codeine-medicated or acetaminophen-medicated mothers during breastfeeding (Chapter 2).

Codeine, a weak opioid analgesic is commonly prescribed for the management of pain related to childbirth partially due to the perception that it has negligible effects on the breastfeeding infant. However, the first report of fatality of an infant following codeine use during
breastfeeding forced regulatory agencies to reassess its safety. Due to increasing reports of codeine intoxication in nursing infants, young infants and adults, some physicians have now started prescribing oxycodone as an alternative for codeine for the management of postpartum pain without concrete evidence to support this recommendation. Thus it was important to determine if the replacement of codeine by oxycodone was safe for both the mother and the breastfeeding infant.

The results of this study showed that:

- The maternal report of neonatal CNS depression in infants of breastfeeding mothers taking oxycodone was similar to those mothers ingesting codeine but higher than those taking acetaminophen.
- Several important clinical features may help identify those nursing infants at risk of experiencing oxycodone intoxication: oxycodone dose used, duration of oxycodone use and maternal CNS depression.
- Oxycodone should not be considered to be a safe alternative for codeine during breastfeeding for the mother-infant pair.

These results demonstrate that oxycodone should be not assumed to be a safer alternative to codeine as one in five infants breastfed by an oxycodone-medicated mother experience symptoms of CNS depression when used for the treatment of maternal pain. Interestingly, mothers who were taking oxycodone were significantly more likely to complain of CNS depressive adverse effects as compared to those who were taking codeine, suggesting that oxycodone should not be assumed to be a safer alternative than codeine for the mother herself. Furthermore, recognition of several important features of this potentially fatal adverse reaction provides evidence in support of the potential use of these factors in identifying neonates at risk for adverse effects as a result of oxycodone exposure via breast milk. This is in agreement with previous studies that have demonstrated that there was higher concordance between CNS depression in the mother and her baby following codeine use (Madadi et al., 2009b; Sistonen et al., 2012), severe CNS depression in breastfed infants became apparent following greater than 4 days of codeine use (Koren et al., 2006; Madadi et al., 2009a; Madadi
et al., 2009b) and mothers of symptomatic infants were using a significantly higher dose of codeine compared to those breastfeeding asymptomatic infants (Madadi et al., 2009b).

This is the first study to date that evaluated the neonatal safety of maternal oxycodone use during breastfeeding. Neonatal safety following maternal oxycodone use has not been systematically evaluated prior to this study and was based on two case reports (Sulton-Villavasso et al., 2012; Timm, 2013) and one small study (n=50) that did not evaluate neonatal outcome following oxycodone exposure (Seaton et al., 2007). The use of oxycodone as an alternative solution for obstetric pain during the postpartum period in women who are breastfeeding is a relatively new practice and deserves much attention in order to minimize the risk of adverse effects in the neonate. It is not surprising that the incidence of neonatal CNS depression following oxycodone exposure via breast milk was similar to that reported by mothers taking codeine because oxycodone is a more potent analgesic compared to codeine, with 28 times higher affinity for the MOR than codeine (Volpe et al., 2011). When comparing the potency of the metabolite of oxycodone to codeine, oxymorphone has a 3-fold increased affinity for the MOR compared to morphine (Volpe et al., 2011). Regardless if it is the parent opioid, oxycodone or its metabolite, oxymorphone, or both that is responsible for mediating the central intoxication in these nursing infants, it cannot be assumed to be safe for breastfeeding infants.

This was a retrospective study consisting of three cohorts (breastfeeding mother-infant pairs exposed to oxycodone, codeine, or acetaminophen-only). The acetaminophen cohort was deemed critical to account for non-specific features that may resemble neonatal CNS depression especially when they are based on maternal reports. This study design not only allowed for the comparison of the incidence of neonatal CNS depression between the oxycodone and codeine cohorts but also comparison of the incidence of maternal CNS depression between the two cohorts, which has not been previously investigated. It was important to not only collect neonatal outcome but also maternal outcome following oxycodone exposure. The fact that mothers taking oxycodone were 17 times more likely to experience CNS depressive adverse effects in addition to other side effects known to be associated with opioid use compared to those using codeine is indicative of the potential
danger associated with the use of oxycodone for the management of obstetric pain for the mother. Nevertheless, the results of the study suggest that physicians need to balance the risks and benefits for the mother-infant pair before prescribing oxycodone for the pain management during the breastfeeding period.

II. Determining the contribution of maternal genetic polymorphisms in drug metabolizing enzymes, transporters and receptors involved in the metabolism and response of oxycodone with respect to maternal and neonatal CNS depression following oxycodone use during breastfeeding (Chapter 3).

The metabolism and response to oxycodone varies widely among patients. Although oxycodone’s analgesic effect is less dependent on the activity of CYP2D6, the contribution of the CYP2D6 genotype in predicting efficacy and avoiding toxicity in the breastfeeding mother-infant pair is currently unknown. This study investigated the role of maternal genetic variants in CYP2D6, CYP3A5, ABCB1 and OPRM1 in oxycodone toxicity in mothers and infants.

The results of this study revealed that:

- Mothers carrying at least one copy of the ABCB1 2677 T variant had an increased risk of experiencing CNS depression.
- None of the studied maternal genetic variants in CYP2D6, CYP3A5, ABCB1 and OPRM1 were associated with neonatal CNS depression.
- Previous clinical factors identified to be predictors of neonatal CNS depression remained significant: duration of oxycodone use and maternal CNS depression.

These results demonstrate that the interplay among clinical factors and maternal genotypes may protect the breastfed infant from sedative outcomes. None of the maternal genetic variants of interest were associated with oxycodone-induced CNS depression in neonates or mothers alone. This may be attributed to the analgesic potency of the parent drug, oxycodone. Thus oxycodone may have been responsible for mediating oxycodone-induced CNS depression in infants and mothers and not necessarily oxymorphone. Although mothers of symptomatic infants were equally as likely to be CYP2D6 UMs as those nursing asymptomatic infants, the CYP2D6 UM mother of symptomatic infant was using a higher dose of oxycodone.
for longer period of time and not supplementing her feeding with formula compared to the CYP2D6 UM mothers of non-CNS depressed infants.

This is the first study to date that investigated if genetic markers in the oxycodone pathway can provide predictive assessment of oxycodone toxicity in susceptible populations, such as mothers and their infants. Although none of the genetic markers alone were predictive of neonatal oxycodone toxicity, this finding uncovers an important limitation of personalized medicine, the inability to make predictions about an individual’s phenotype based only on genetic variations. These predictions may be more powerful when they combine genetic information with clinical information, as shown previously with codeine intoxication (Madadi et al., 2009b; Sistonen et al., 2012). Future research is required to explore the joint effect of maternal genetic variants and clinical factors possibly through mechanistic modeling to predict both maternal and neonatal oxycodone intoxication. Nonetheless, the results of this study are encouraging and highlighting their potential utility in identifying mothers and infants at risk for opioid-related adverse effects.

III. Determining the ontogeny of P-gp in endothelial cells of the developing human BBB (Chapter 4).

The greater sensitivity to the central depressive effect of morphine may be attributed to the limited expression of P-gp at the neonatal BBB (Bouwmeester et al., 2003; Koren et al., 1985; Way et al., 1965). The mechanism underlying this heightened sensitivity that may lead to life threatening adverse events has not been fully explored from the fetal up to the postnatal period. This study assessed the ontogeny of P-gp in the developing human BBB.

The results of this study revealed that:

- There was a progressive increase in the intensity of P-gp immunoreactivity from GA 20 weeks to PNA 0-3 months, with the greatest increase between PNA 0-3 and 3-6 months ($p<0.05$).
- Levels of P-gp do not reach adult levels by term indicating further postnatal changes, reaching adult levels by 3 to 6 months of life.
These findings uncovered a possible mechanism underlying the life-threatening opioid
toxicity seen with maternal codeine use during breastfeeding. Following the death of the
neonate who was breastfed by a CYP2D6 UM mother using codeine, several groups of
researchers questioned the interpretation of results (Bateman et al., 2008; Ferner, 2008).
Briefly, points such as postmortem changes in morphine concentrations, infant milk intake
leading to morphine poisoning and CYP2D6 UM phenotype was not always predictive of
morphine intoxication have been raised. Since the distribution of morphine across the BBB is
influenced by activity of P-gp, differences in BBB P-gp expression during brain development will
likely mediate its analgesic and adverse effects. These results provide further evidence to
support the heightened sensitivity of neonates to morphine as previously shown in both animal
(Bragg et al., 1995; Kupferberg and Leong Way, 1963; Rai et al., 2005) and human studies
(Bouwmeester et al., 2003; Koren et al., 1985; Way et al., 1965).

This is the first study that investigated the ontogeny of P-gp in the developing human
brain from the late second trimester to 6 months of age. Previous animal and human studies
only characterized the ontogeny of BBB P-gp up to term and showed that its expression
increases with advancing age (Daoed et al., 2008; Ek et al., 2010; Schumacher and Mollgard,
1997; Tsai et al., 2002; Virgintino et al., 2008). Since P-gp expression in term infants was still low
compared to adults (Daoed et al., 2008), it was important to describe the developmental
expression of P-gp during the postnatal period and determine the age at which P-gp reaches
maturation in the infant. Due to the limited expression of P-gp at birth, neonates may be at risk
for CNS drug toxicity. As P-gp expression reaches adult levels by 3 to 6 months of age, older
infants have the capacity to efflux morphine from the brain before it has a chance to exert its
depressant effect. The implication of this finding is not restricted to morphine, but can be
extrapolated to other P-gp substrates that are prescribed to mother-infant pairs or directly to
neonates. Many drugs that are prescribed in pediatrics are P-gp substrates and act within the
brain. These include but are not limited to cancer chemotherapeutics (daunorubicin, docetaxel,
etoposide, placlitaxel), antiepileptics (phenobarbital, phenytoin) and HIV protease inhibitor
(ritonavir). Understanding the ontogeny of P-gp from the fetal up to the postnatal period would
be useful for predicting the efficacy when drug delivery to the fetal or newborn brain is beneficial and risk for adverse events with drug use in the young.

IV. **Assessing the relationship between genetic polymorphisms and drug interactions as related to codeine and morphine concentrations (Chapter 5).**

Genetic polymorphisms and drug interactions can influence the parent opioid and metabolite concentrations, which likely influence the efficacy and safety of opioids. This study investigated whether genetic variations in the codeine metabolic pathway and drug interactions affect the codeine and morphine concentrations in codeine-related deaths in Ontario, Canada.

The results demonstrated that:

- Deaths deemed suicidal were associated with higher codeine concentrations than those that were deemed accidental (455 ng/mL vs. 315 ng/mL, respectively; $p<0.05$).
- The presence of other CNS depressants significantly influences codeine and morphine femoral blood concentrations ($p<0.05$).
- The M/C ratio was modulated by the presence of a CYP2D6 inhibitor at varying potencies.
- Deceased individuals who carried the $ABCB1\ 1236T$ variant had significantly lower morphine femoral blood concentrations than the wild-type carries ($p<0.05$).

From this work, it is apparent that concomitant use of codeine with other centrally acting drugs is more toxic than codeine used alone. Concomitant use of opioids with other CNS depressants appears to be common in this cohort, providing evidence on how and why codeine-related deaths occur. Thus this combination should be avoided or at least closely monitored. Understanding the patterns and characteristics amongst codeine-users whose cause of death was related to opioid toxicity and how they contribute to toxicity are necessary in order to develop holistic and multifaceted preventative strategies (Madadi et al., 2013). Furthermore, the strength of a CYP2D6 inhibitor used concomitantly with codeine and the manner of death significantly modulated the codeine and morphine concentrations. Therefore, several factors such as genetic polymorphisms, concomitant CNS depressants and drug interactions affect the M/C ratio, leading to discordance between the CYP2D6 genotype to
phenotype predictions. This study demonstrates the central challenge of personalized medicine in terms of predicting the efficacy and safety of codeine using genetics alone. An individual’s genotype only reflects the genetic makeup but not the current state of an individual. A study conducted by Sistonen and colleagues found that the combination of genetic and clinical factors better predicted CNS depression symptoms in mothers and their infants following codeine use during the postpartum period compared to models including only clinical or genetic factors (Sistonen et al., 2012). Thus, combining genetic and non-genetic information will likely yield more accurate predictions of an individual’s response to codeine and risk for codeine intoxication.

Due to ethical limitations, it is difficult to study the toxicological effect of drug interactions in human studies. This makes these postmortem cases valuable as they assist in the interpretation of drug concentrations in deaths involving opioid intoxication. Given the high incidence of multirug toxicity in this unique and powerful cohort of deceased individuals, the results have the potential to influence prescribing-decisions and assist in the interpretation of drug concentration in deaths involving codeine intoxication.

2 Limitations and Future Directions

The results in this thesis provide compelling evidence that CNS depressive adverse drug, which can be predicted by several factors, can occur in the breastfeeding mother-infant pair. Despite this knowledge, physicians are likely going to continue prescribing opioids specifically codeine and oxycodone for postpartum pain relief. This is attributed to the fact that these two opioids are much better studied in breastfeeding women compared to other analgesic alternatives. Therefore, they must be prescribed under the emerging direction of personalized medicine in order to maximize its efficacy while minimizing the risk for life-threatening adverse events.

Identifying genetically susceptible patients via pharmacogenetic testing prior to oxycodone administration is likely going to prevent CNS depression in these patients (Chapter 3). Similar genetic approaches in this thesis have modestly identified various variants in drug metabolizing enzymes and targets in the oxycodone pathway leading to differences in response
and risk of toxicity in both human and animals without any consistent conclusions. Although these findings are clinically intriguing, the genetic data necessary to support the use of pharmacogenetic testing is currently lacking. In order to achieve this goal, large genome-wide association studies of individuals are needed in order to elucidate pharmacogenomic influences on oxycodone pharmacokinetics and pharmacodynamics and thereby aid in identifying genetically susceptible patients at risk for opioid intoxication. It is likely that two or more genes may have joint effects and that variations in these genes together may better predict opioid response and safety. Therefore, large human clinical studies are needed to explore the joint effects of genetic variants in predicting opioid intoxication. Successful incorporation of a pharmacogenetic test into routine clinical practice not only requires reliable results but also effective translational strategies. Future validation of whether pharmacogenetic testing will be a cost-effective strategy for improving the efficacy and safety profile of opioid use is required.

From this thesis, several potential predictive factors have been identified that merit further evaluation. As determined from the research presented in this thesis (Chapters 3 and 5), it is unlikely that any of these factors alone will predict CNS depression following opioid use. Rather the interplay between genetic variants in the opioid pathway and non-genetic factors (clinical factors, ontogeny and drug interactions) will likely better predict opioid efficacy and safety. Whole-body physiology-based pharmacokinetic (PBPK) modeling is a promising method to describe the action of a drug in the population of interest while coupling it with factors that may influence its absorption, distribution and elimination to identify high-risk parameter combinations for drug intoxication (Bjorkman, 2005; Edginton et al., 2006). Critical parameter combinations that increase the risk for morphine poisoning in breastfed neonates following repeated codeine administration to the breastfeeding mother were identified using coupled PBPK models for the mother and child (Willmann et al., 2009). Given that neonates have reduced morphine clearance at birth, potentially toxic morphine plasma concentrations can be reached within 4 days in the neonate following repeated codeine dosing to either a CYP2D6 UM or EM mother (Willmann et al., 2009). A similar approach to include the genetic and non-genetic predictors identified from this thesis should be taken in the future to identify high risk combinations for opioid intoxication for both the mother and the infant.
Also, future work is necessary to further understand the mechanisms involved in infant susceptibility to opioid-induced toxicity. This research showed that the expression of P-gp in the brain is under developmental influence, which may contribute to susceptibility to opioid-induced toxicity (Chapter 4). There is virtually no data on the contribution of genetic polymorphisms on P-gp mediated drug transport in newborns. Recent studies have suggested that hepatic drug metabolizing enzymes such as CYP2D6 and UGT are also developmentally regulated. More importantly, CYP2D6 and UGT expression has been detected in the brain, which may contribute to cerebral opioid metabolism (Nagano et al., 2000). The ontogeny of these drug metabolizing enzymes in the human fetal brain warrants further investigation.

3 Conclusions

Several pieces of evidence are presented in this thesis regarding the potential use of predictors to assess the risk for opioid intoxication in this susceptible population. First, this research showed that oxycodone is not a safe replacement for codeine for obstetric pain and identified several clinical factors such as maternal dose, duration of oxycodone use and maternal CNS depression. Second this research identified a genetic marker, the ABCB1 2677 T variant, that was associated with maternal opioid intoxication, thereby providing additional evidence in support of the use of genetic biomarkers to identify those at risk for this life-threatening adverse event. Third, this research demonstrated that the developmental expression of P-gp can contribute to variability in the disposition of opioids in the brain and hence susceptibility to opioid intoxication. Lastly, this research revealed the patient’s phenotype can be modulated by the presence of other CNS depressants and drug interactions. This has important implications with respect to increasing the complexity in genotype to phenotype predictions of an individual’s opioid response and risk for adverse events. In summary, the results presented in this thesis fill several knowledge gaps that existed with respect to explaining the high interindividual variability for the risk of opioid intoxication and contribute to new knowledge that will help bring personalized medicine for pain management into clinical practice.
4 References


Appendices

Appendix A. Data Collection Document for Oxycodone Cohort Study (Chapter 2).

![Breastfeeding Follow Up Form](image)

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</tbody>
</table>

Hello, my name is __________. May I please speak to __________. I am calling from the Motherisk program located at the Hospital for Sick Children. You may remember you called Motherisk in __________ for some information about oxycodone during breastfeeding. We are now doing a follow-up interview in women who used oxycodone during breastfeeding. It will take about 5 to 10 minutes and of course, everything will be confidential.

Would you like to help us with our study? ☐ No ☑ Yes
Did you end up taking oxycodone while breastfeeding? ☐ No ☑ Yes
If no, do you mind telling me why? ________________________________

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Specify: ________________________________
Neonatal Health
Health in intensive care unit? □ No □ Yes Home at: _______ days

Breastfeeding with the Use of Oxycodeone
Are you currently still breastfeeding while taking oxycodeone? □ No □ Yes (if yes move below. If no move to "if no longer breastfeeding")

If Still Breastfeeding with the Use of Oxycodeone
How often are you breastfeeding? # breastfeeds: _______/day (or every ________ hrs)
How long is the baby fed each time? ____________________________
Weight of mother during breastfeeding? ____________________________
Have you introduced formula? □ No □ Yes
When did you introduce formula? _______ mths # formula feeds:______/day (____ oz each)

If No Longer Breastfeeding or Not Using Oxycodeone
Are you currently still breastfeeding? □ No □ Yes
When did you stop breastfeeding? ____________ mos age
Why did you stop breastfeeding or use oxycodeone during breastfeeding?
□ concerned for baby □ went to work □ went to cows milk □ did not need to take oxycodeone anymore □ other: __________________________

Within a 24 hour period how often did you breastfeed while you were still taking oxycodeone?
# breastfeed: _______/day (or every ________ hrs)
How long was the baby fed each time? ____________________________
Weight of mother during breastfeeding with oxycodeone? ____________
Age of child when still breastfeeding with oxycodeone? ____________________________ Weight: ____________________________
Have you introduced formula? □ No □ Yes
When did you introduce formula? _______ mths # formula feeds:______/day (____ oz each)

Was codeine ever prescribed to you before, after, or during the time you were taking oxycodeone? □ No □ Yes: specify when: __________________________

Medications

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| 2         |            | ongoing □ |      |          |
|           |            |           |      |          |
| indication:|            |           |      |          |

| 3         |            | ongoing □ |      |          |
|           |            |           |      |          |
| indication:|            |           |      |          |

| 4         |            | ongoing □ |      |          |
|           |            |           |      |          |
| indication:|            |           |      |          |
Did you use any vitamins or herbal preparations? □ no  □ yes

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</table>

Adverse Events (check one of the provided choices or write down mom’s exact words)

When you were taking oxycodone, did you notice any changes in yourself? □ yes □ no
□ drowsiness/sedation/increase sleep □ nausea □ vomiting □ constipation □ stomach pain
□ dizziness □ weakness □ other:

Did you notice any physical or behavioural changes in your baby? □ yes □ no
□ drowsiness/sedation/increased sleep □ irregular breathing □ vomiting □ constipation
other:

If Adverse Event in Baby

How long after you started using oxycodone did this happen? □ min □ hr □ d
How long did it last? □ min □ hr □ d
Did you consult the child’s doctor? □ yes □ no What did the doctor say?
What did you do? □ no change □ stopped taking medication □ stopped breastfeeding
□ other:

Results:

When you stopped taking the drug did you notice an improvement in the sleepiness?

Within a 24 hour period, what was the longest time your baby would sleep uninterrupted during the time you were on oxycodone?

What is normal? Typically babies would wake up between feeds.

Maternal Race:

Please specify the country your grandparents came from:

□ Caucasian: country □ Black: country
□ Indo-Asian: country □ Hispanic: country
□ Oriental-Asian: country □ First Nations: country
□ Middle Eastern: country
□ Other details:

1 Explanation: we want to see if the baby’s response will differ as a result of the mother’s ethnic background. This brings me to the second part of the study.

2 If Canadian: have you done a family tree or know where your grandparents or ancestors immigrated from.
Consent to Saliva and Breast Milk Collection

Saliva Collection:
There is one more thing I would like to ask you. The reason we are doing this study is to gather more information about the safety of oxycodeone during breastfeeding. When we take oxycodeone, it is broken down in our body into active compounds that help with pain relief. This breakdown is controlled by genes, and different people have different numbers of copies. We want to see if the number of copies a mother has and other variations involved in pain medication response is related to how the mother or baby feels with the use of oxycodeone. We would be able to look at this by just collecting a saliva sample from you that we can send to you over the mail. All you need to do is sign a consent form, spit into the tube and send it back to us. All this will be free of charge. We would be happy to call you back with the results of the study as well if you are interested.

Would you like to donate your saliva to us? □ No □ Yes

If yes: may I have your full name and address so I can send you the study package? Page 1
You should receive the study package within the next 2 weeks with the consent form and instructions. If you have any questions please call me at (416) 813-7283. In the meantime, thank you for helping us out with our study and we will keep in touch with you.

If no: Thank you, Ms. _______ for helping us out with our study and have a good day.

Breast milk collection:
In addition, since you are currently still breastfeeding while using oxycodeone, we are interested in measuring the level of oxycodeone in breast milk. We can send you some collection cups for this. We need no more than a couple of tablespoon of breast milk. We will also send you a tracking log where you can indicate the time you took oxycodeone and collected the breast milk. You can keep the cups in your freezer and we will arrange to have them picked up for analysis.

Are you interested in collecting some breast milk for us? □ No □ Yes

How much to collect?
You can collect as many as you feel comfortable with at different times of the day as long as you indicate the time you took oxycodeone and the time you collected the breast milk. We can send you 5 collection cups if you want and for each cup you can collect a couple tablespoons of breast milk (approximately 10ml).

What is the concern for long term use of oxycodeone for my child?
The effects of oxycodeone are only short term. So we don’t expect any long term effects such as interference with brain or physical development. It is also worthy to note that oxycodeone and opioids are used during surgery and prescribed for post-operative analgesic in children. We are only interested in the acute effects of oxycodeone.
Appendix B. Research Consent for Genetic Testing of Saliva Sample (Chapter 3).

Research Consent for Genetic Testing

Principal Investigator:
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  416-813-5781

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  416-813-7654 ext 4413
- Jessica Lam, BSc: jessica.lam@sickkids.ca
  416-813-7283

I confirm that Jessica Lam has explained the genetic test that I am about to have done with respect to genes involved in opioid (codeine, oxycodone) response, and that any questions that I have asked have been answered to my satisfaction. The discomforts, consequences and possible risks associated with these tests have been explained to me. I understand that it is my choice whether or not to have this testing. Results of this test will be explained to me and I understand that this information may be shared, if necessary, with professionals involved in my/my child's medical care, including our family physician. I have been assured that records relating to me or my child and the care that we received will be kept confidential, and that no information will be released or printed that will reveal my or my child's identity without my permission or unless required by law.

I understand that the interpretation of the genetic information will depend in part on the family information that I have given. Differences between family information and the results of genetic tests occur when the parents of a child are different from those reported. No paternity may be detected with this testing.

I understand that although genetic testing is usually accurate, as with all testing some inaccuracies may occur. Also genetic testing is ongoing and new research may mean that the interpretation of the test results may change over time. On occasion, in the process of testing for one genetic condition, another genetic alteration may be identified. Such findings would be reported to your health care provider to discuss with you. I understand that it is my responsibility to notify the Clinical Pharmacology department of any change of address, and to check with the department for updated genetics information and counseling that I feel I may need, for example in making decisions about a pregnancy.

I understand that if I apply for insurance and provide consent, information in my medical records, including the results of genetic testing will be available to the company. My sample may also be used so that other research may be done, but only after all identifying information, like my name has been removed (see open consent only for this type of research).

Consent Form Version: June 17, 2010
Pages 1 of 2
Closed Consent:
Closed consent means that any tissue or DNA obtained from me or my child will be analyzed and then destroyed. Specifically, I give my consent for a blood/tissue sample to be taken for testing related only to CYP2D6, UGT 2B7, COMT, OPRM1, and MDR1 genotype testing; this testing will be undertaken in an accredited clinical service laboratory and/or a research laboratory and that the sample and any DNA extracted from it will be destroyed once the results of the testing are available. I also understand that if I want any further genetic testing to be done in the future, I will need to have another sample taken from me or my child.

Signature: ______________________  Date: ______________________
Witness: _______________________  Date: ______________________
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