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A Pilot Study of the Pharmacogenetics of Ketamine-Induced Emergence Phenomena: A Dissertation

Edwin N. Aroke

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A PILOT STUDY OF THE PHARMACOGENETICS OF KETAMINE-INDUCED
EMERGENCE PHENOMENA

A Dissertation Presented

By

EDWIN N. AROKE

Submitted to the Faculty of the
University of Massachusetts Graduate School of Nursing, Worcester
In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Nursing

April 21, 2016

University of Massachusetts Worcester

Graduate School of Nursing


A Pilot Study of the Pharmacogenetics of Ketamine-Induced Emergence Phenomena

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By

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DEDICATION

This work is dedicated to family:

To my Mother of Blessed memory, Mama Margaret Anu Aroke, who taught me to believe in my dreams, and my father, Pa Johnson Aroke, who exemplified humility, hardwork and discipline in his life;

To my wife, Magdaline Aroke, who has been a constant source of support and encouragement throughout this journey;

To my children, Belinder, Edwin, Ethan, Erin, and Emily who are the source of my greatest joy and inspiration;

To God Almighty for giving me the strength and endurance to follow my dreams

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Special thanks to the GSN leadership and Professor Charles Vacchianno (Duke University) who helped fund my sample processing. You believed in me, when no one did. Drs Singla Sudershan and Steve Heard, thank you for critiquing my work. Dr Hilary Aroke and all my siblings you challenged me to dream bigger and has been a constant source of inspiration. I will like to thank my fellow students, especially Jessica Pegano-Therrein, Michel Griswold, and Susan Natale, for their continual support and friendship throughout this program, I couldn't have asked for better friends. To Mami Theresia Anu and Christelle Afoawung, thank you for being there.

Finally, I am grateful to my colleagues in the UMMHC Department of Anesthesiology and the SACU/PACU Nurses at Hahnemann and Memorial Campuses, who cheered and encouraged me during my dissertation. Your encouragement kept me going.

ABSTRACT

Background: Up to 55% of patients administered ketamine, experience an emergence phenomena (EP) that closely mimics schizophrenia and increases their risk of injury. While genetics accounts for about 50% of severe adverse drug reactions, no studies have investigated genetic association of ketamine-induced EP in healthy patients. Ketamine is metabolized by CYP2B6 enzymes and CYP2B6*8 allele significantly alter ketamine metabolism. In addition, ketamine exerts most of its effects by inhibiting the N-methyl-D-aspartate receptor (NMDAR), and NMDAR genes (*GRIN2B*) are associated with learning and memory impairment and schizophrenia.

Purpose: To investigate the relationship between *CYP2B6*6* and *GRIN2B* single nucleotide polymorphisms (SNPs) and ketamine-induced emergence phenomena (EP).

Methods: This cross-sectional pharmacogenetic study recruited 75 patients having minor orthopedic, hand, foot, anorectal surgeries from two outpatient surgical centers. EP was measured with the Clinician Administered Dissociative State Scale (CADSS). DNA was genotyped using standard Taqman assays and protocols. Genetic association of *CYP2B6*6* and *GRIN2B* (rs1019385 & rs1806191) SNPs and ketamine induced EP occurrence and severity were tested using multivariate logistic and linear regression, adjusting for age, ketamine dose, duration of anesthesia, and time since ketamine administration.

Results: Forty-seven patients (63%) received ketamine and were genotyped. Nineteen EP cases were identified (CADSS > 4), leaving 28 non-EP controls. For our population, CADSS has an internal consistency reliability Cronbach's alpha of 0.82, and could reliably distinguish ketamine from non-ketamine cases. Occurrence and severity of EP were not associated with *CYP2B6*6* or *GRIN2B* ($p > 0.1$). Models removing genotype and containing age, ketamine dose, duration of

anesthesia, and time since ketamine administration significantly predicted EP occurrence ($p = 0.001$) and severity ($p = 0.007$). Presence and severity of EP did not affect patient satisfaction with care.

Discussion: Younger age, higher dose and longer duration of anesthesia significantly predicted EP occurrence and severity among our sample. This study provides effect size estimates useful for the design of adequately powered future genetic association studies. The feasibility of recruitment from patients undergoing elective, outpatient surgeries and ease of post-operative EP assessment with CADSS supports our approach. However, the small sample size may have limited about ability to determine significant differences.

Conclusion: Fully powered studies are needed to investigate this important phenomena. Determining factors for anesthesia-related EP symptoms may reduce risks and costs associated with this adverse medication effect.

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Running Head: PHARMACOGENETICS OF KETAMINE

Pharmacogenetics of Ketamine Induced Emergence Phenomena: Dissertation Proposal

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EXPLORING THE PHARMACOGENETICS OF KETAMINE-INDUCED EMERGENCE PHENOMENA

Ketamine is an N-methyl-D-aspartate receptor (NMDAR) antagonist that is recognized by the World Health Organization (WHO) as an essential drug in any basic health care system (World Health Organization, October 2013), and has been used in various settings from battlefields (Dickey, Jenkins, & Butler, 2012), developing countries (Morgan & Curran, 2012) to critical care units in tertiary care centers (Mohrien, Jones, MacDermott, & Murphy, 2014). It is an ideal anesthetic because it causes rapid loss of consciousness, amnesia, analgesia, cardio-pulmonary stability, and treatment of mental disorders (Aroni, Iacovidou, Dontas, Pourzitaki, & Xanthos, 2009; Mion & Villevieille, 2013; Zarate, Brutsche, Ibrahim, et al., 2012; Zarate, Brutsche, Laje, et al., 2012). Unfortunately, ketamine-induced emergence phenomena (EP) which occurs in up to 55 percent of patients (Kumar, Bajaj, Sarkar, & Grover, 1992) limits its clinical use (L. J. Mason, 2004). EP is characterized by euphoria, vivid dreams, illusions, delirium, and hallucinations (Aroni et al., 2009; Stoelting & Hillier, 2006; Xu & Lipsky, 2014). Patients experiencing EP are at higher risk of injury, longer hospital stay, require more nursing care, and report lower satisfaction with care (Hudek, 2009; O'Malley, 2014; Stoelting & Hillier, 2006; Wells & Rasch, 1999). In addition, EP increases cost to patients, providers, and the health care system (Franco, Litaker, Locala, & Bronson, 2001). Research efforts to date have focused on identifying pharmacological interventions to prevent EP (Stoelting & Hillier, 2006). There is no definitive treatment or predictive tools to identify patients who will develop EP.

While several factors contribute to individuals' response to drugs, it is believed that genetic predisposition accounts for about 50 percent of severe adverse drugs reactions (ADRs) (Chidambaran, Ngamprasertwong, Vinks, & Sadhasivam, 2012; Hijazi & Bouliou, 2002;

Yanagihara et al., 2001). Single nucleotide polymorphisms (SNPs), which are the most common type of genetic polymorphisms, have been associated with alterations in drug metabolism, efficacy, and response. Two genes of interest in this phenomenon are *CYP2B6* and *GRIN2B*. Ketamine is metabolized primarily by the enzymes encoded by *CYP 2B6* (Hijazi & Boulieu, 2002; Portmann et al., 2010; Yanagihara et al., 2001), and in-vitro studies have shown that SNPs in *CYP2B6**6 significantly alters its metabolism (Y. Li et al., 2013). The signs and symptoms of EP closely mimic those of schizophrenia and ketamine is frequently used as a pharmacologic model for this disorder. Ketamine exerts most of its central nervous system (CNS) effects through the N-methyl-D-aspartate receptor (NMDAR), and its effects on this pathway are the bases for the glutamatergic theory of schizophrenia (Javitt, 2010). Genetic polymorphisms in the NMDAR genes, like *GRIN 2B*, have been associated with learning and memory impairment, and schizophrenia (Frohlich & Van Horn, 2014; Xu & Lipsky, 2014). Thus, I hypothesize that ketamine-induced EP may be related to genetic variations in *CYP2B6* and *GRIN2B*.

The purpose of this exploratory study is to investigate the relationship between *CYP 2B6*, and *GRIN2B* polymorphisms and the incidence and severity of ketamine-induced EP in healthy patients undergoing scheduled ambulatory surgical procedures. The specific aims of this study are:

Aim 1: Explore genetic association between *CYP2B6* SNPs and ketamine-induced EP. As common functional *CYP2B6**6 SNPs affect ketamine metabolism, I hypothesize genetic association with risk alleles of *CYP2B6**6 and greater incidence and severity of EP. Leukocyte DNA and Clinician Administered Dissociative State Scale (CADSS) will be used to determine genetic polymorphisms and the incidence and severity of EP, respectively. Potential effect modification by midazolam will be explored.

Aim 2: Explore genetic association between *GRIN2B* SNPs and ketamine-induced EP. Because ketamine is an NMDAR antagonist that induces glutamate release in the CNS and glutamate hypo-function correlates with psychotic symptoms, EP mimics schizophrenia (Frohlich & Van Horn, 2014) . Moreover, given that *GRIN2B* SNPs are associated with schizophrenia, learning, and memory disorders (Demontis et al., 2011; D. Li & He, 2007), I hypothesize that patients with common SNPs in *GRIN2B* will develop similar psychological responses to ketamine. Potential effect modification by midazolam will be explored.

Aim 3: Explore if *GRIN2B* genes interact with *CYP 2B6* in predicting the incidence and severity of ketamine induced EP. Given that SNPs in *CYP2B6**6 allele alter ketamine metabolism, and SNPs in *GRIN2B* alter NMDAR function, I hypothesize that patients who rapidly metabolize ketamine and have decreased NMDAR function will have reduced incidence and severity of EP.

Aim 4: Evaluate the validity of the CADSS in discriminating EP behaviors associated with ketamine.

The correlation between the incidence and severity of ketamine-induced EP, *GRIN2B* and *CYP2B6* genetic polymorphism in healthy patients has not been studied. An *a priori* knowledge of patients that will develop ketamine-induced EP based on their genetic make-up may have significant future implications for anesthesia care, procedural sedation, acute and chronic pain management (including cancer pain), and management of mental disorders. This study will provide preliminary data necessary for the development of a program of research to improve our understanding of risk for EP and identify genetic predisposition to EP, both of which could lead to improved patient outcomes. Advancing the nurse anesthesia profession through pharmacogenetic research is my dream.

BACKGROUND AND SIGNIFICANCE

Clinical Uses of Ketamine

First introduced into clinical practice about 50 years ago (Domino, 2010), ketamine is an N-methyl-D-aspartate receptor (NMDAR) antagonist that is used for induction of anesthesia, procedural sedation, pain management and treatment of major depression (Aroni et al., 2009; Mion & Villevieille, 2013; Zarate, Brutsche, Ibrahim, et al., 2012; Zarate, Brutsche, Laje, et al., 2012). The NMDAR is a heteromeric voltage-gated ionotropic receptor containing two NR1 subunits in association with two NR2 (A, B, C, or D) and/or NR3 (A or B) subunits. Activation of this receptor requires glycine (an inhibitory neurotransmitter) and glutamate (an excitatory neurotransmitter). Ketamine non-competitively blocks the excitatory effects of glutamate (its principal neurotransmitter) by binding to the NR2 subunit (Monaghan & Jane, 2009). This leads to depression of sensory association in the cerebral cortex, limbic system, and thalamus, resulting in rapid loss of consciousness (Aroni et al., 2009).

Unlike other anesthetics such as propofol, patients under ketamine-induced loss of consciousness maintain spontaneous respiration and protective airway reflexes. In addition, its sympathomimetic effects lead to hemodynamic stability and bronchodilation. Thus, it is the preferred anesthetic in patients with severe asthma (Lau & Zed, 2001) and hemodynamic instability such as hypovolemia, cardiogenic shock and constrictive cardiac disorders (Aroni et al., 2009). It is equally the sedative of choice in situations where airway management equipment is not readily available (e.g. battlefields (Dickey et al., 2012), developing countries (Morgan & Curran, 2012), endoscopy and radiology suites (K. P. Mason et al., 2002).

Despite the fact that ketamine works on other receptors, most of its effects are on the NMDAR. The NMDAR has a significant role in acute and chronic pain management (Monaghan

& Jane, 2009). Ketamine has an opioid sparing effect. A Cochrane review show that ketamine decreases post-operative opioid requirement (Bell, Dahl, Moore, & Kalso, 2006) and it is a potential breakthrough for management of opioid refractory cancer pain (Bell, Eccleston, & Kalso, 2012; Bredlau, Thakur, Korones, & Dworkin, 2013). It also effective in the management of phantom limb pain (Alviar, Hale, & Dungca, 2011), fibromyalgia, and chronic regional pain syndrome (Correll, Maleki, Gracely, Muir, & Harbut, 2004).

Ketamine is administered be several routes: intravenous, subcutaneous, intramuscular, per-rectal, sub-lingual, epidural and inhalations. It is therefore a good induction drug for uncooperative patients, children, and patients with burns who have limited IV access (Aroni et al., 2009). Its bioavailability via the oral route is low (about 20%) because ketamine undergoes extensive first pass metabolism. It is metabolized primarily by the hepatic cytochrome p450 2B6 (CYP2B6) enzyme with minor contributions from CYP3A4 and CYP2C9 (Hijazi & Boulieu, 2002; Yanagihara et al., 2001). However, renal and hepatic functions do not appear to significantly affect its metabolism and elimination (Mion & Villevieille, 2013). Thus, I hypothesized that genetic alternations in the structure and function of the primary drug-metabolizing enzyme (CYP2B6) affect the incidence and severity of ketamine induced EP.

Ketamine Induced Emergence Phenomena

Ketamine-induced emergence phenomena (EP) refers to the psychological (psychodysleptic or psychedelic) effects experienced by some patients as they recover from ketamine induced loss of consciousness (Curran & Morgan, 2000; Mion & Villevieille, 2013; Morgan & Curran, 2012). The incidence of EP varies from 5 to 55 percent (Kumar et al., 1992; White, Way, & Trevor, 1982b) and it remains the main reason for ketamine's limited clinical use (L. J. Mason, 2004). Risk factors of EP include age>15 years, doses >2mg/kg IV, rapid

administration, female gender, and mental disorders (O'Malley, 2014; Sdrales, 2013; Stoelting & Hillier, 2006). EP is a significant adverse drug reaction because it prolongs hospital length of stay, increases risk of injury to patients and staffs, and increases nursing and medical staff requirements (Hudek, 2009). In addition, patients who experience EP report decreased satisfaction with care, and incur additional cost (Uezono et al., 2000). It is equally costly to the institution and the entire health care system (Franco et al., 2001).

Nature of Ketamine Induced Emergence Phenomena

Ketamine-induced emergence phenomena (EP) is frequently used interchangeably with ketamine-induced delirium (Kumar et al., 1992; Lohit, Srinivas, & Chanda-Kulkarni, 2011; Treston et al., 2009). While ketamine induced emergence phenomena includes the wide range of psychological symptoms (from euphoria to combative hallucination) reported by patients following ketamine administration, ketamine induced delirium is more restrictive in that it describes patients who experience agitation, restlessness and combative behavior without being aware of their surroundings (Treston et al., 2009; Uezono et al., 2000). EP is characterized by euphoria, floating sensation, vivid dreams, disorientation, auditory and visual illusions, which may progress to delirium and hallucinations and a sense of dissociation from the environment (Cunningham & McKinney, 1983; Domino, 2010; Siegel, 1978). Clinically, patients appear semi-conscious (eyes open), and breathe spontaneously with random movements, but are unable to process and respond to sensory and noxious stimuli.

Several studies have investigated the nature of ketamine-induced EP. Using a psychonautic approach (a methodology of describing and explaining the subjective effects of drugs), Newcombe (2008) described a “ketamine trip” to the “ketamine world”. In the ketamine world (also known as K- world), subjects experience “strange things [that] appear to be giving

information ...with distinct auditory hallucinations” (p. 212). The psychonaut described a sense of dissociation from the environment, an euphoric, energetic state with visual and auditory distortions that lasted about an hour or two (Newcombe, 2008). A phenomenological study of recreational users of ketamine, found that ketamine induced distortions in perceptions similar to negative symptoms of schizophrenia: flat affect, poverty of speech, thought disorders and delusions but did not cause hallucination (Pomarol-Clotet et al., 2006). Pomarol-Clotet and colleagues (2006) described distortions in body image and objects (sharpness, color and size), which some authors have attributed to hallucinations (J. H. Krystal, Karper, Seibyl, & et al., 1994). In fact, Krystal and Colleagues (1994) found that sub-anesthetic doses of ketamine produced cognitive impairment, positive and negative psychotic symptoms. As a result, ketamine is used as a pharmacological model for schizophrenia (Frohlich & Van Horn, 2014).

Using a single photon emission tomography (SPET) scan of healthy volunteers with highly specific NMDAR ligand, Stone and Colleagues (2008) discovered that ketamine produced negative psychotic symptoms by binding to the NMDAR, while the positive psychotic symptoms were related to ketamine blood levels. However, they did not find any relationship between positive and negative psychotic symptoms (Stone et al., 2008). In a subsequent study using magnetic resonant imaging, Stone and colleagues (2012) described ketamine-induced the release of glutamate from the anterior cingulate cortex. The ketamine induced release of glutamate correlates with positive psychotic symptoms (Stone et al., 2012). A comprehensive review of the literature on ketamine as a model for schizophrenia found that ketamine-induced EP correlates with negative and positive psychotic symptoms of schizophrenia (Frohlich & Van Horn, 2014).

Current Management/Prevention of EP

Physical and chemical restraints have been used to manage EP (Wells & Rasch, 1999). Benzodiazepines such as midazolam are frequently co-administered with ketamine to prevent EP (Cartwright & Pingel, 1984; White, Way, & Trevor, 1982a). Several randomized controlled trials comparing midazolam-ketamine against ketamine alone did not find any significant difference (Sherwin, Green, Khan, Chapman, & Dannenberg, 2000; Wathen, Roback, Mackenzie, & Bothner, 2000). More recently, a double blind randomized placebo-controlled trial found that co-administration of midazolam significantly reduced the incidence of EP (Sener, Eken, Schultz, Serinken, & Ozsarac, 2011). But unlike the Sherwin and Colleagues (2000) study that reported the psychometric properties of their measurements, Sener and colleagues (2011) did not utilize any developed instruments to measure EP. They measured EP “as any moaning, screaming, cursing, unpleasant dreams, or unpleasant hallucinations, regardless of severity” (Sener et al., 2011, p. 110), some of which could be signs of pain not EP. Unfortunately, no pain scores were reported. Thus, the co-administration of midazolam with ketamine remains a reasonable option since midazolam does potentiate amnesia and decrease anxiety (Green, Roback, Kennedy, & Krauss, 2011).

Other drugs that have been investigated to prevent/manage EP and found to have mixed results include haloperidol (Amr, Shams, & Al-Wadani, 2013), dexmedetomidine (Levanen, Makela, & Scheinin, 1995) and propofol (Alletag, Auerbach, & Baum, 2012; Andolfatto & Willman, 2010). The use of music to attenuate ketamine-induced EP, did not show any significant difference (Kumar et al., 1992). To date, there is neither a definitive treatment for EP, nor a clinical tool to predict which patient will develop EP. As Edward Domino eloquently described ketamine “beware of that tiger... it needs to be tamed” (Domino, 2010, p. 683).

CONCEPTUAL FRAMEWORK

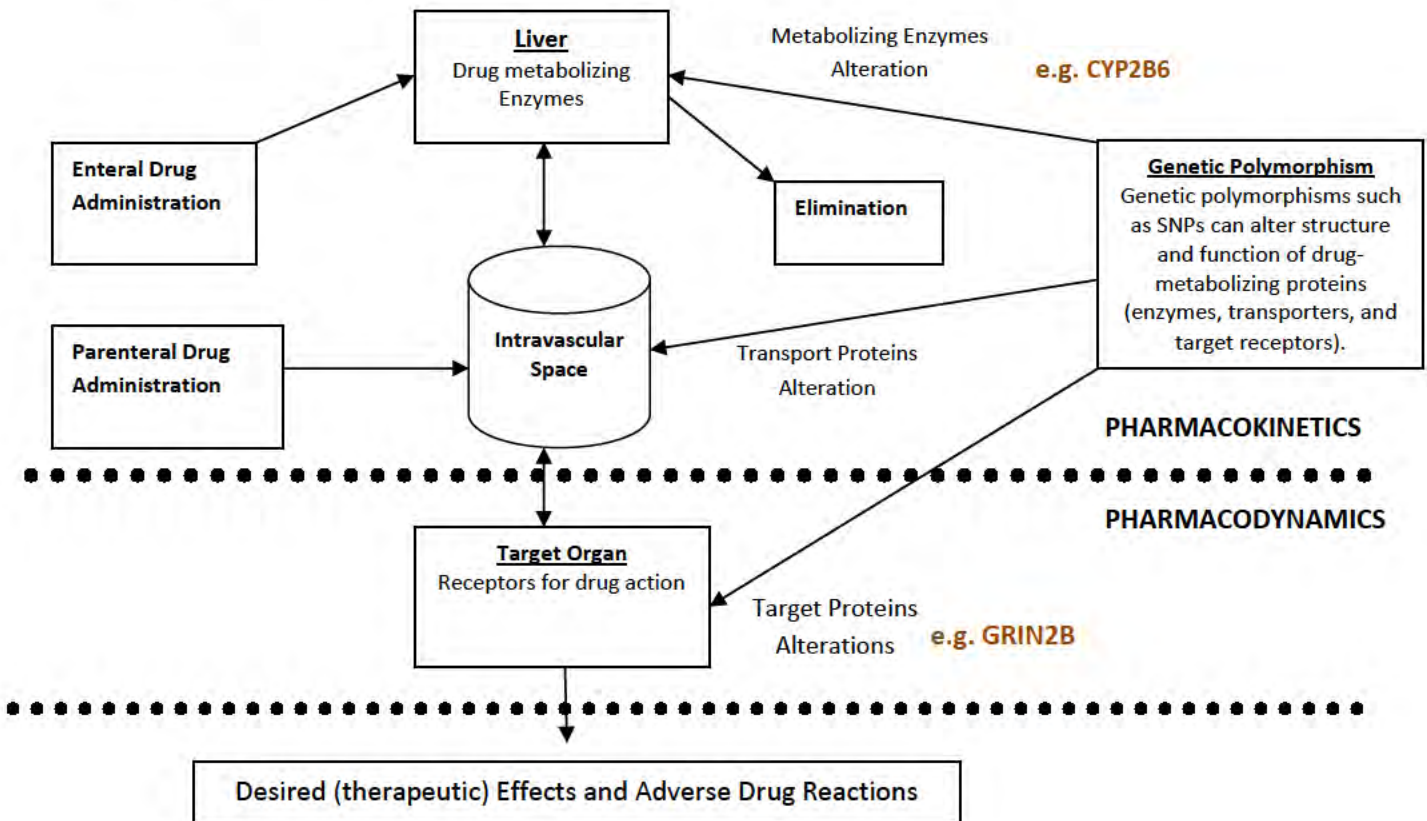
Genetic bases of variability in drug response

Historically, clinicians have depended on the pharmacological properties of drugs (such as size of molecules, charge, solubility, concentration, volume of distribution, and mode of excretion) to predict patients response to medications. And despite tremendous advances in our knowledge of pharmacology, patients are still at risk for suffering adverse drug reactions due to inter-individual variability in their response to drugs (Wang, 2010). While several factors influence individuals' response to medications, genetic predisposition accounts for about 50 percent of severe adverse drug reactions (SADRS) (Chidambaran et al., 2012). The genetic base of inter-individual variability in drug response was first established when Kalow and colleagues (1957) discovered that an atypical form of the enzyme responsible for metabolizing succinylcholine (butyrylcholinesterase, also known as plasma or serum cholinesterase) was responsible for prolonged apnea after succinylcholine administrations. In 1959, Vogel coined the term "pharmacogenetics" to describe the research that combines our knowledge of pharmacologic properties of drugs and genetic variability to determine onset, duration, emergence, and response to medications (Altman, Flockhart, & Goldstein, 2012).

Advances in molecular biology have shown that variation in nucleotide base sequences on DNA, can result in alterations in the structure and function of proteins. The most common of these genetic variations are single nucleotide polymorphisms (SNPs). SNPs occur in more than 1 percent of the population and result when fragments of DNA differ between individuals by one specific nucleotide in a DNA sequence. SNPs that result in amino acid changes (during translation), are referred to non-synonymous variations, while those that do not alter the amino acid are referred to as synonymous variations. Genetic variations (SNPs) that result in non-

synonymous amino acid substitution are of significant interest in pharmacogenetics research because they alter the structure and function and proteins. As shown in figure 1, genetic alterations in the structure and function of target proteins, drug metabolizing enzymes (proteins) and drug transport proteins account for some of the observed inter-individual variability in response to medications.

Figure 1. Pharmacogenetic Conceptual framework



Pharmacokinetics variability:

Pharmacokinetics is the study of drug disposition (absorption, distribution, metabolism, and excretion). Thus, pharmacokinetic variability deals with “variability in the delivery of a drug or metabolite(s) to target molecules” (Roden & George Jr, 2002, p. 37). Alterations in drug transport proteins and drug metabolizing enzymes, affect their delivery to target proteins.

Enzymes variants that increase drug metabolism will decrease the availability of parent drug at the target site, while increasing the availability of its metabolite. Similarly, enzyme variants that decrease metabolism will increase the concentration of the parent drug, while decreasing the accumulation of its metabolites. These have implications for the development of adverse drug reactions (Altman et al., 2012; Roden & George Jr, 2002; Wang, 2010).

As mentioned above, ketamine is metabolized primarily by enzymes coded by *CYP2B6* with minor contributions from *CYP 3A4* and *CYP 2C9* (Hijazi & Boulieu, 2002; Yanagihara et al., 2001). *CYP2B6* has many SNPs that alter drug action (U. M. Zanger & K. Klein, 2013). Specifically, *CYP2B6*6* haplotype has two SNPs that result in non-synonymous amino acid substitutions, Gln172His (rs3745274, c.516G>T) and Lys262Arg (rs2279343,785A>G) in exons 4 and 5 respectively (U. M. Zanger & K. Klein, 2013). *In vitro* studies show that *CYP2B6*6* allele significantly affects ketamine metabolism (Y. Li et al., 2013). Similarly, SNPs in the same allele (*CYP2B6*6*) significantly alter the N-demethylation of methadone (another NMDAR antagonists) (Dennis, Bawor, Thabane, Sohani, & Samaan, 2014; Gadel, Crafford, Regina, & Kharasch, 2013; Kharasch & Stubbert, 2013) and increases CNS side effects in HIV-infected patients treated with efavirenz (Gounden, Van Niekerk, Snyman, & George, 2010). However, among patients with treatment resistant depression and bipolar disorder, Zarate and colleagues (2012) did not find any relationship between *CYP alleles* and EP (Zarate, Brutsche, Laje, et al., 2012). History of mental disorder is a risk factor of EP (Stoelting & Hillier, 2006), and to date, no study has investigated the correlation between *CYP alleles* and EP in healthy patients.

Pharmacodynamics variability:

The term pharmacodynamic variability refers to “the relationship between the drug concentration and its effects” (Roden & George Jr, 2002, p. 39). Individuals with the same

concentration of drug in blood and target tissue have different responses to the drug. Such individual differences can be accounted by variability in tissue (target protein) response to the drug (Altman et al., 2012; Daly & Arranz, 2012; Roden & George Jr, 2002).

Ketamine binds to the NR2 subunit of the NMDAR, and blocks the action of glutamate. Functional MRI studies have shown that it induces glutamate release in a manner similar to its release in NMDAR hypo-function (Frohlich & Van Horn, 2014) . NMDAR hypo-function and glutamate hyperactivity are the molecular bases of schizophrenia (Javitt, 2010). In addition, genetic polymorphisms in the glutaminergic pathway have been associated with NMDAR hypo-function and symptoms of schizophrenia, learning, and memory disorders (Frohlich & Van Horn, 2014; D. Li & He, 2007; Xu & Lipsky, 2014). Two SNPs (T200G (rs1019385), 4197T/C (D. Li & He, 2007) and rs1806194 (Demontis et al., 2011)) in the NMDAR gene (*GRIN2B*) have been linked to schizophrenia in susceptible individuals. Thus, ketamine-induced EP may be related to NMDAR- NR2B subunit function.

RESEARCH APPROACH

Design

An exploratory cross sectional design with candidate gene association approach will be utilized to investigate the relationship between *CYP2B6* and *GRIN2B* polymorphisms and ketamine-induced EP in relatively healthy patients undergoing anorectal, orthopedic, hand or foot surgery.

Sample and Setting

Participants in this study will comprise a convenience sample of 135 patients presenting for ambulatory hand or foot surgery at the University of Massachusetts Memorial Health Care: University, Memorial, and Hahnemann Campuses. The power to detect statistical significance depends on the number of SNPs, allelic frequencies of each SNP, effect size and study design (Ross, Anand, Joseph, & Pare, 2012, p. 9). Based on additive and dominant genetic models of inheritance, a sample of 135 with a population allelic frequency of 25%, 80% power, and 0.05 significance, can detect a relative risk of 2 (Schmidt, 2007). This detectable relative risk of 2 converts to an approximate odd ratio of 2 (Zhang & Yu, 1998), because the incidence of EP in patients not exposed to ketamine is expected to be low (< 10%). Thus, this sample size is powered to detect an EP association with an observed odd ratio of about 2.5 during procedural sedation (Elia & Tramer, 2005). Results from this study may inform larger studies investigating this important phenomenon

Limited difficulty is anticipated in obtaining a sample of 135 patients because these two facilities perform over 2000 hand and foot surgical procedures yearly on relatively healthy patients. The PI is employed as a Certified Registered Nurse Anesthetist at this hospital and has

letters of support from the Chair of the Anesthesia Department, the Perioperative Medical Directors, and Directors of Perioperative Nursing Services.

Table 1

Inclusion and Exclusion Criteria with Rationale

Inclusion	Rationale
ASA Class 1 and 2	Focus on healthy patients
Read and write English	Unable to provide interpreter in multiple languages
Adult 18 years and older	Children are less likely to experience EP (Frohlich & Van Horn, 2014)
<u>Exclusion</u>	<u>Rationale</u>
History of mental disorder	Increase risk of psychological effects (Stoelting & Hillier, 2006)
Allergic to Ketamine	Ensure participants safety
Contraindications to ketamine e.g. uncontrolled hypertension, history of ketamine addiction	Sympathomimetic effects of ketamine can increase blood pressure - Prevent possible relapse in participants
Currently taking Rifampicin, Dilantin or Phenobarbital	-CYP2B6 enzyme inducers(Ulrich M Zanger & Kathrin Klein, 2013) may alter the ketamine metabolism
Pregnancy	The safety of ketamine during pregnancy has not been established.

Procedures

Once Institutional Review Board (IRB) approval is obtained from the University of Massachusetts Worcester, the PI will communicate specifics of the study plan to all involved parties including the Chair of the Anesthesia Department, Directors of Perioperative Services, and Nurse Managers. This letter will review the purpose of the study and the procedures necessary to collect data. The letter will be followed with a presentation to members of the anesthesia department (Anesthesiologists and CRNAs) during regularly scheduled weekly grand round meetings. All providers will be given the chance to ask questions about the study and study pamphlets will be distributed. A similar meeting will be held with Surgical Admissions Care Unit (SACU) and Post-Anesthesia Care Unit (PACU) nurses at both campuses. All nurses will be given the chance to ask questions about the study and study pamphlets distributed. The study has been approved by all hand and foot surgeons. A HIPAA waiver will be obtained from the IRB to access the list of potential participants scheduled for surgery and their pre-operative assessment. The pre-operative assessment will be used to verify inclusion/exclusion criteria. Potential participants will be given a handout describing the study during their preoperative visit at the pre-surgical evaluation (PSE) clinic. On the eve of the scheduled surgical procedure, the PI will call potential participants to discuss the study.

When the potential participants present for surgery they will undergo routine pre-procedure assessment by the SACU nurse and anesthesia provider. After this, the PI will discuss the study with them and review all components of the consent form, including the study purpose, procedures, potential risks and benefits. The PI will allow time to answer all questions and willing participants will be asked to read and sign the IRB-approved informed consent form. Consenting participants will be asked to complete a paper and pencil demographic form with

information about their age, race, smoking history, alcohol use, highest level of education and gender. Completed forms will be returned to the PI. Preoperative sedative will be administered at the discretion of the anesthesia provider. Standard of care allows for many alternative anesthetics to be used to achieve intra-operative sedation. The anesthesia provider will determine the choice of anesthetic.

Clinician Administered Dissociative State Scale (CADSS)

Ketamine-induced EP is most likely to occur within one hour of emergence from anesthesia (Sdrales, 2013). Thus, within one hour of participants' arrival in the PACU, the PI will administer the CADSS, a validated instrument that assesses a dissociative state. This scale contains 28 (23 subjective and 5 objective) items followed by 5 anchors. The anchors are rated from 0 (no symptoms) to 4 (extreme symptoms). A total score of ≥ 4 on CADSS signifies a dissociative state (Bremner, 2014). Initial psychometric testing reported an inter-rater reliability of 0.92 ($p < .01$), an internal consistency Cronbach's alpha of 0.94 ($p < .05$), and a construct validity correlation of 0.48 and 0.42 with Dissociative Experiences Scale (DES) and Structured Clinical interview for DSM-IV-R-Dissociative Disorder (SCID-D) respectively (Bremner et al., 1998). CADSS has been used in many studies investigating the psychological effects of ketamine (Curran & Morgan, 2000; Dakwar et al., 2014; J.H. Krystal et al., 1998; Pomarol-Clotet et al., 2006; Radant, Bowdle, Cowley, Kharasch, & Roy-Byrne, 1998; Zarate, Brutsche, Laje, et al., 2012). In this study, two additional questions (items 29 and 30) will be added in an attempt to capture the participants' overall impression of the anesthetic.

Measures, Data Collection and Laboratory Methods

The PI will review the patient record and collect data on perioperative medications and dosages after completion of the CADSS. The PI will abstract data on height, weight, substance abuse, current medications and dosages from the medical record. Patients who received additional sedatives such as propofol, opioids, haldol, and droperidol will be used as control cases to determine the discriminatory validity of CADSS for ketamine-induced EP.

All patients who receive ketamine will have two vials of 5ml venous blood collected in an EDTA tube, prior to the removal of the intravenous line. If aspiration of the IV access fails to return blood, a direct venous puncture will be used. One vial will be used for genotype, while the second vial will be stored for future studies. Each blood tube will be labeled with a study ID number in front of the participant. Leukocyte DNA is stable in EDTA at 4°C for 72hours. Samples will be frozen on ice and transported to UMass core laboratory, where it will be stored frozen at -20 degrees centigrade until analyzed. The freezer will be locked, and only the PI and laboratory manager will have access to the specimen. A hand-written table containing coding system (study ID number and patient's name) will be kept in a locked filing cabinet. Only the PI will have access to this cabinet. This will be the only source of data to match the participants to the study ID.

Genetic Analysis

Genomic DNA for SNP identification will be extracted using QIAamp DNA Blood mini kit (Qiagen, USA). When ready for analysis, the blood samples will be delivered to a UMass based molecular biology core laboratory per standard biohazard transfer procedures. Samples will be thawed and DNA extracted and purified according to the manufacturer's instructions. DNA will be quantified and assessed for quality using Nanodrop spectrophotometers. *CYP 2B6*

SNPs (rs3745274, and rs2279343) and *GRIN2B* SNPs (rs1019385 and rs1806191) alleles will be genotyped using Taqman Genotyping Assays (Applied Biosystems) (see Table 2. Gene/SNPs).

The laboratory personnel will be blinded to the participants' demographic information and phenotype. All samples will be run against control standards and the genotype call rate/error rate obtained and reported. As an additional control, 10% of randomly selected samples will be re-genotyped for each of the genes. All unused samples will be stored in a locked freezer after data analysis at UMass.

Table 2

Gene/SNP variants

Gene	SNP (Polymorphic/ mutation)	Functional change	Chr	Location*	Alleles (minor/ wild- type)	MAF (HapMap)
<i>CYP2B6</i> *6	rs3745274 (missense)	Gln172His	19	41006936	T/G	27% (Cau), 42% (Yrb)
	rs2279343 (missense)	Lys262Arg	19	41009358	G/A	21% (Cau), 46% (Yrb)
<i>GRIN2B</i>	rs1019385 (polymorphic)	--	12	13981909	T/G	50% (Cau), 3% (Yrb)
	rs1806191 (synonymous)	His440His	12	13563704	A/G**	44% (Cau), 84% (Yrb)**

SNP, single nucleotide polymorphism; Gln, glutamine; His, histidine; Lys, lysine; Arg, arginine; Cau, Caucasian/European; Yrb, Yoruban/African American

*NCBI Build GrCh38 (base pair location of SNP)

**Minor allele for rs1806191 is G; any race-stratified analyses will account for the G allele as the minor variant.

Statistical Analysis

Data will be imported into SPSS (version 21) for analysis. Double entry will be used to ensure accuracy. Demographic data and genetic allele frequencies will be analyzed and presented

as means (continuous variables) and frequencies (categorical variables). Descriptive statistics (frequencies, means, standard deviations, and percentages) will be calculated for all study variables as appropriate to the level of data. For continuous variables, mean, median, skewness, standard error of the mean, standard deviation, and histograms will be calculated. Frequencies will be run on all categorical variables. All continuous variables will be checked for normal distribution by calculating Fisher's measure of skewness. Internal consistency reliability will be evaluated using Cronbach's alpha as appropriate.

The occurrence of ketamine-induced EP will be analyzed as a categorical variable, determined by a total CADSS score of ≥ 4 , per the instruments' scoring criteria for EP. Severity of ketamine-induced EP will be analyzed as a continuous variable (total score on CADSS). Leukocyte genomic DNA will be used to determine participants' genotypes for *CYP2B6* and *GRIN2B* genes. Each genetic marker (SNP) will consist of a biallelic locus. For this purpose, alleles will be represented *d* (wild type allele) and *D* (minor or risk allele). Possible genotypes will be *d/d*, *d/D* and *D/D*. All SNPs will be tested for independence and stability in the population using the Hardy-Weinberg Equilibrium (HWE) equation.

Aims 1 and 2: Investigate the genetic association between SNPs and incidence and severity of Ketamine-induced EP.

All hypotheses are two-tailed and tested at a 0.05 significance level. Multiple testing will be controlled with Bonferroni correction. All self-reported racial/ethnic groups will be analyzed together, except for analyses involving rs1806191, as the minor allele is opposite in Yoruban/African American populations, requiring sub-stratified analysis of this variant by race.

All SNPs will be evaluated independently, using both additive and dominant genetic models. Both the dominant and additive genetic models use tests with one degree of freedom,

which are generally more powerful than a wide range of genetic models. The additive model tests the effect of each risk allele in the SNP in a linear fashion, such that having 1 risk allele (d/D) has a greater effect than having zero risk alleles (d/d), and having 2 risk alleles (D/D) shows a greater effect than having only 1 (d/D). We will employ chi-square 2 x 3 contingency table to compare additive genotype counts (d/d , d/D , DD) against EP status (positive EP occurrence, no EP). Results will be expressed as odds ratios of the incidence of EP by genotype status.

The dominant model tests the effect of having at least one copy of the risk alleles in the SNP on the occurrence of EP, such that genotypes d/D and d/d are combined and compared to wild-type homozygous (D/D) individuals. A chi-square 2 x 2 contingency table will be used compare genotype counts (d/d and d/D vs. D/D) and EP status. Results will be expressed as odds ratios of the incidence of EP by genotype status (carriers of d vs. DD). To determine whether genotypes predict severity of EP, linear regression models will test the additive and dominant genetic effects on the total CADSS score (range: 0-112) in EP incident cases ($CADSS \geq 4$), controlling for age, gender, dose of ketamine, duration of surgery, and use of midazolam.

In order to evaluate the impact of concurrent midazolam administration and other potential confounders on genetic associations with ketamine-induced EP incidence, logistic regressions will also be performed using both additive and dominant genetic models as described previously. All SNPs will be tested independently for their prediction of EP occurrence (0,1), controlling first for midazolam alone, then in separate models controlling for age, sex, dose of ketamine, duration of surgery, and use of midazolam.

Finally, all analyses will be conducted similarly in the non-ketamine exposed participants in order to explore comparisons. This may further aid in distinguishing exposure- and/or genetic-

mediated effects. For example, if significant genetic associations are found in both ketamine- and non-ketamine exposed groups, it may provide preliminary evidence of genetically-mediated EP that is independent of ketamine. Likewise, lack of significant genetic associations in non-exposed groups compared to significant associations in ketamine-exposed groups adds more formal evidence in this pilot design.

Aim 3. Investigate if *GRIN2B* genes interact with *CYP 2B6* in predicting the incidence and severity of ketamine induced EP

To investigate Aim 3, an interaction term *CYP2B6*GRIND2B* genotypes will be added to the logistic and linear regression models described above. Significant Type III Sums of Squares for the interaction term indicates gene-gene interaction effects for incidence of ketamine-induced EP and/or EP severity.

Aim 4. Evaluate the validity of the CADSS in discriminating EP behaviors associated with ketamine use.

The frequency and degree of EP will be compared between the group of patients who received ketamine and those who did not. Significance will be $p < .10$ (two-tailed) for this analysis to reduce the probability of a type II error as a result of the small sample size. When analyzing data from exploratory studies not fully powered it is acceptable to increase the level of significance (Campbell, 2005; Cohen, 1994).

Confounding Variables

Possible confounding variables include age and administration of midazolam. Due to the potential confounding of age, post-hoc analyses will be performed in which the subjects are stratified by age for the Chi-square analyses and comparisons made between the age-specific

odds ratios. Premedication with midazolam prevents the determination of genetic effect due to ketamine alone. Given that many patients receive midazolam preoperatively for anxiolysis, determination of genetic effects of ketamine in the context of premedication with midazolam will produce generalizable results to a real-world clinical scenario. Thus, determination of genetic predisposition to EP, despite the administration of midazolam will closely mimic current clinical practice.

Population stratification always exists as a confounding variable when participants in a genetic association study come from more than one subpopulation that differ in genotype frequency. The HWE will be used to assess for population stratification and any deviation corrected in the statistical analysis.

Potential Problems, Alternate Strategies, and Limitations

If for some reason, the UMass core Laboratory is not available, DNA samples will be transferred per IRB-approved biological transfer protocol, to Duke University's Molecular Core Facility in the Duke Molecular Physiology institute where the PI's primary mentor is based. If recruitment into the study is inadequate, the surgeons will be contacted to introduce the study to the patients. In addition, the study protocol will be expanded to cover procedures performed at other UMass facilities and affiliated hospitals such as UMass Memorial campus, and Marlboro Hospital.

The limitations of this study include that (1) the gene-environment, gene-gene, and drug-drug interactions will not be completely controlled, (2) nonrandom sample will limit generalizability, and (3) the indirect effect of other genes is not investigated. Data analysis will control for all drug administered during the perioperative period, while genetics analysis will include analysis of linkage disequilibrium. In addition, patients taking drugs that are known to

inhibit the CYB2B6 enzyme will be excluded from the study. Given that the application has no *a priori knowledge* of which patients will develop EP or will have a particular candidate gene, the risk of bias due to non random sampling is greatly reduced. In addition, extra effort will be made to recruit a diverse population especially women and minorities.

PROTECTION OF HUMAN SUBJECTS

This study protocol will be reviewed and approved by the University of Massachusetts, Worcester) Institutional Review Board (IRB). Every effort will be made to protect participant's privacy and confidentiality, and minimize risk. Written informed consent will be obtained prior to enrollment and collection of any data. The four essential elements of valid informed consent will be employed in the consent process: a) provision of all relevant information about the study, b) making the sure the information is understood, c) ensuring that potential participants are capable of consenting and d) ensuring that potential participant are not coerced (Vijverberg, Pieters, & Cornel, 2012). The PI has completed CITI training on ethical and responsible research. All data collected will be de-identified, and the investigators will comply with Health Insurance Portability and Accountability Act of 1996 (HIPPA) regulations.

Participants will have the right to withdraw from the study, or decline to answer specific questions at any point. Should the participant become distressed, or uncomfortable during assessments, the assessment will be terminated with the option of completing later left to the participant. The PI will attempt to comfort such participants and provide any helpful explanations. If the discomfort or distress is related to the surgery and not the study assessments, the PI will immediately notify the nurse and/or anesthesia provider.

There are no anticipated physical risks to participants. However, there is a chance that venous blood cannot be obtained from the established IV access. In that instance, venous blood will be obtained by direct veni-puncture. This risk will be included in the informed consent and discussed with participants in advance. Blood samples will be stored in the locked research freezer at UMMS, accessible only to laboratory personnel and the PI. All samples will be stripped of personal identifiers and only the PI will have access to the single master file linking

the research ID number on the sample, data sheets and the participants' name. No additional, genetic profiling will be carried out on the samples, except those stated in this research protocol. Samples from participants who provide additional written consent will be stored for future research studies. Samples from participants who do not provide the additional written consent will be destroyed at the completion of the study. Separate IRB approval will be obtained for future analysis on stored samples.

There is always the potential for breach of confidentiality when study instruments are completed but this risk will be greatly minimized by using unique research ID number. All information provided by the participants will be referenced to a unique research ID number. And all paper data forms will be stored in a secure locked cabinet within a locked office, accessible only to the PI, while electronic data will be stored in a UMASS password protected, encrypted R drive assigned to the PI.

The clinical expertise of the applicant is anticipated to limit the potential risk to participants. All study participants will be monitored for risks related to study participations. No results will ever be reported in a personally identifiable manner, only grouped data will be presented in presentations and publications. The usual standards for medical confidentiality will be observed.

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EXECUTIVE SUMMARY

A pilot study of the pharmacogenetics of ketamine-induced emergence phenomena:

A Dissertation Presented by

Edwin N. Aroke

University of Massachusetts, Worcester

This study explored the relationship between single nucleotide polymorphisms in the cytochrome p450 (*CYP 2B6*) and N-Methyl-D-Aspartate receptor (*GRIN2B*) genes, and the incidence and severity of ketamine-induced emergence phenomena (EP) in relatively healthy patients. Table 3 below summarizes the changes made to the original research proposal approach and rationale for the changes.

Table 3

Summary of changes to dissertation proposal

Original Proposal	Change	Rationale for the Changes
Title: Exploring the Pharmacogenetics of Ketamine-Induced Emergence Phenomena Sample size of 135	Exploring the Pharmacogenetics of Ketamine-Induced Emergence Phenomena: A Pilot Study Sample size of 75	The title was changed to better reflect the nature of the study. In addition, sample size for reduced. Sample size was reduced because of cost, time constraints and lack of pilot data to justify the full study.
Participants will include healthy patients undergoing hand or foot surgery.	Participants included patients undergoing minor orthopedic, hand, foot and anorectal procedures.	Increasing the number of surgical procedures potential participants were recruited from, reduced recruitment time and time needed to complete the study.
On the eve of the scheduled surgical procedure, the PI will call potential participants to discuss the study.	No potential participants were called on the eve of the surgical procedure.	Calling of potential participants was discouraged by IRB staff.
When the potential participants present for surgery they will undergo routine pre-procedure assessment by the SACU nurse and anesthesia provider. After this, the PI will discuss the study with them and review all components of the consent form, including the study purpose, procedures, potential risks and benefits.	When the potential participants presented for surgery they underwent routine pre-procedure assessment by the SACU nurse and anesthesia provider. <i>At the end of the assessment, the nurse asked potential participants their willingness to discuss the study with the PI.</i> The PI discussed the study with willing potential participants, and reviewed all components of the consent form, including the study purpose, procedures, potential risks and benefits	This additional step was necessary to ensure that only willing potential participants were approached by the PI. This gave them the chance to opt-out of the study even before meeting the PI. This was a vital step in the consent process because the PI works in the same facility as a Nurse Anesthetist. It ensured that participation all aspects of the study was completely voluntary.
Consenting participants will be asked to complete a paper and pencil demographic form with information about their age, race,	All forms (demographic form, CADSS scale, and Data collection sheets) were completed on all subjects on a secured web-based	Use of REDCaps reduced potential errors that could be introduced during transcription of pencil and paper surveys. Data can be verified easily by

smoking history, alcohol use, highest level of education and gender

Standard of care allows for many alternative anesthetics to be used to achieve intra-operative sedation. The anesthesia provider will determine the choice of anesthetic.

Samples will be frozen on ice and transported to UMass core laboratory, where it will be stored frozen at -20 degrees centigrade until analyzed. DNA extraction and genotyping will be at a UMASS based molecular biology laboratory.

application: REDCaps, and downloaded onto a UMass secure drive that is backed up daily.

Standard of care allows for many alternative anesthetics to be used to achieve intra-operative sedation. The anesthesia provider determined the choice of anesthetic.

However, if the provider chose to use ketamine, they administered the standard dose of 0.5mg/kg. To ensure patient safety and comfort other medications were administered at the discretion of the anesthesia provider.

Samples were stored at -20 degrees for about one month, then moved to -80 degrees freezer in batches for long term storage.

DNA extraction was performed at a UMASS based laboratory.

The extracted DNA was shipped to Duke University where the genotyping was done at the Duke Molecular Physiology Institute.

Committee chair and data can be downloaded easily to SPSS for analysis.

The dose of 0.5mg/kg of ketamine is typically administered as an adjunct for pain management and during sedation.

Standardizing the dose helped control the effect of dosage on EP.

DNA is more stable at -80 degrees than -20 degrees centigrade.

The cost of genotyping was much lower at Duke University compared to UMASS.

DISSERTATION DEFENSE SLIDES

A Pilot Study of the Pharmacogenetics of Ketamine-Induced Emergence Phenomena:

A Dissertation

Edwin Aroke

April 21st 2016

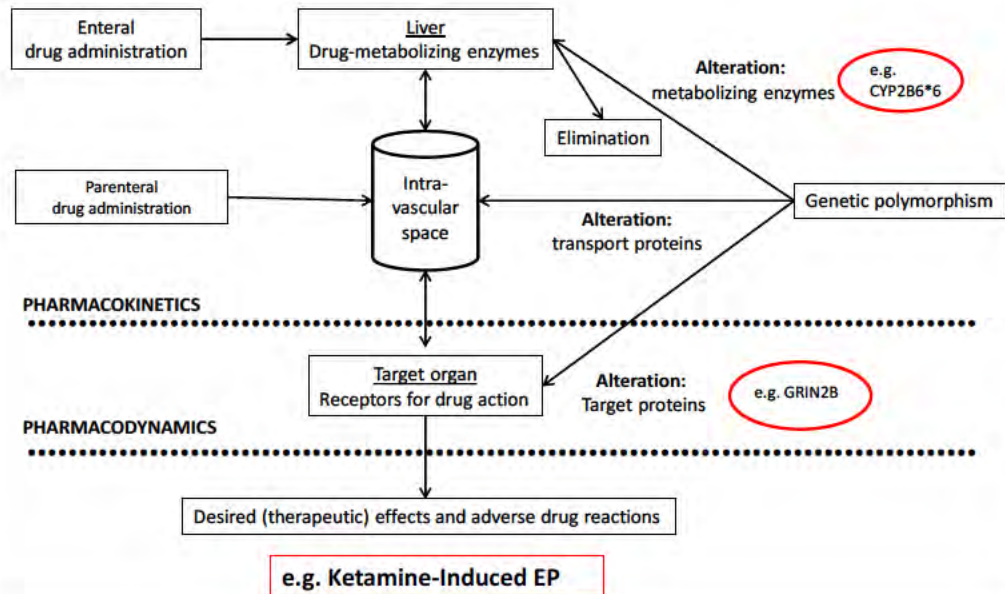
Graduate School of Nursing

University of Massachusetts Worcester

Background and Significance

- Up to 55% of patients administered ketamine experience Emergence Phenomena (EP)
 - EP is characterized by dissociative symptoms and mimics schizophrenia
 - EP may increase risk of injury, prolong hospital stay, increase nursing care requirement and cost, and lower satisfaction.
- Genetics account for about 50% of severe adverse drug reactions

Pharmacogenetic Conceptual Framework



The Gap

- No genetic association studies of ketamine-induced EP in otherwise healthy patients.

Purpose

- To investigate genetic association between *CYP2B6* and *GRIN2B* single nucleotide polymorphisms (SNPs) and the incidence and severity of ketamine-induced EP in healthy patients

Specific Aims

1. Explore genetic association between *CYP 2B6* SNPs and ketamine-induced EP
2. Explore genetic association between *GRIN 2B* SNPs and ketamine-induced EP
3. Evaluate effect of ketamine-induced EP on patient satisfaction with anesthesia
4. Determine effect size of ketamine-induced EP
5. Evaluate the accuracy of Clinician Administered Dissociative State Scale (CADSS) in discriminating ketamine-induced EP from other post-anesthetic behaviors.

Methods

- Cross sectional candidate gene pharmacogenetic association study
- IRB approval was obtained from UMMS IRB

Inclusion and Exclusion Criteria

Inclusion

- ASA 1 &2
- Read and write English
- Adults 18 years and older
- Scheduled for minor orthopedic, hand, foot or anorectal surgery with sedation

Exclusion

- History of depression, bipolar disorder and schizophrenia
- Contraindications to ketamine e.g. uncontrolled hypertension, history of ketamine addiction
- Current use of Rifampicin, Dilantin or Phenobarbital
- Pregnancy

Procedure

Informed Consent

Procedure

CADSS

Blood Collection



Genetic Analysis

- Venous blood in EDTA tubes
 - Stored at -80 degrees Celsius
- Genomic DNA extraction using QIAgen protocol
 - Purity assessed by spectrophotometry (OD at 260nm)
- Genotyping using standard Taqman Genotyping Assays
 - 2 non-template and 2 known template controls
 - 2 samples randomly re-genotyped

Genotype

Gene	SNP (Polymorphic/mutation)	Functional change	Chr	Location*	Alleles (minor/wild-type)	MAF (HapMap)
<i>CYP 2B6</i>	rs3745274 (missense)	Gln172His	19	41006936	T/G	27% (Cau), 42% (Yrb)
	rs2279343 (missense)	Lys262Arg	19	41009358	G/A	21% (Cau), 46% (Yrb)
<i>GRIN 2B</i>	rs1019385 (polymorphic)	--	12	13981909	T/G	50% (Cau), 3% (Yrb)
	rs1806191 (synonymous)	His440His	12	13563704	A/G**	44% (Cau), 84% (Yrb)**

Phenotype

Clinician Administered Dissociative State Scale (CADSS) determines diagnosis & severity of EP

- EP diagnostic phenotype: CADSS \geq 4
- EP severity phenotype: total CADSS score (range 0-112)

Statistical Analysis

- SPSS Version 22
- Student's t, Mann-Whitney U , chi square & Fisher's exact tests
- Logistic and Linear Regression
- $P < 0.1$ was statistically significant
- Hardy Weinberg Equilibrium (HWE) & Dominant Genetic Model

dd versus Dd or DD

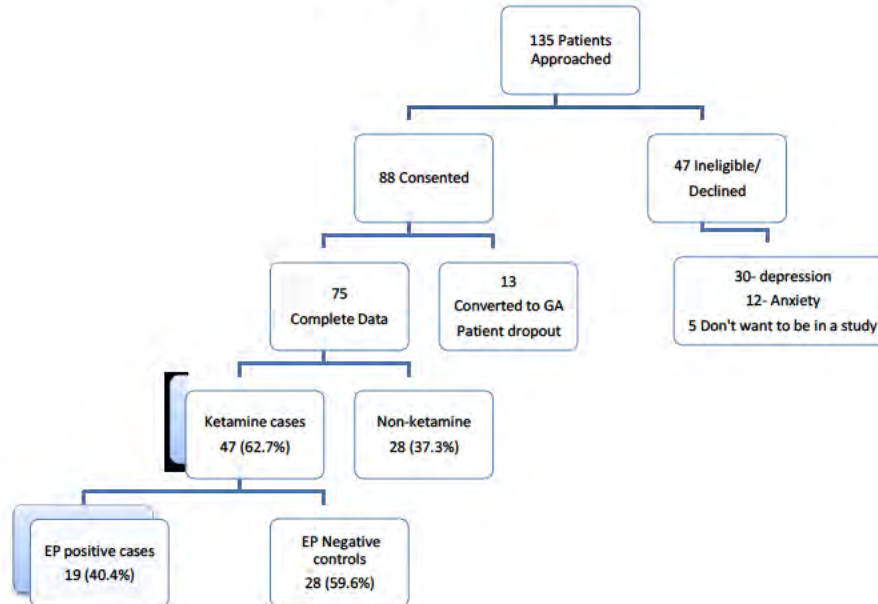
Alleles

- d= wild type
- D= risk allele

Genotypes

- dd
- Dd
- DD

RESULTS



**Characteristics of study participants:
No demographic differences between various groups**

	No Ketamine	No EP Ketamine	EP Ketamine	P-values ^a	P-values ^b
	N = 28 (37.3%)	N = 28 (37.3%)	N = 19 (25.3%)		
Characteristics		Mean (SD)			
Age (years)	51.89 (13.57)	51.04 (13.02)	47.84 (16.04)	0.522	0.456
Body Mass Index (kg/m²)	29.99 (6.61)	27.23 (5.43)	28.28 (4.98)	0.095	0.503
Gender		N (%)			
Males	11 (39.3)	15 (53.6)	9 (47.4)	0.323	0.676
Female	17 (60.7)	13 (46.4)	10 (52.6)		
Race					
White or Caucasian	22 (78.6)	26 (92.9)	16 (84.2)		
Asian	1 (3.6)	1 (3.6)	1 (5.3)	0.455	0.651
Black or African American	2 (7.1)	1 (3.6)	1 (5.3)		
More than one Race	1 (3.6)	0	0		
Unknown/Not Reported	2 (7.1)	0	1 (5.3)		
Ethnicity (N, %)					
Hispanic	3 (10.7)	2 (7.1)	2 (10.5)	0.599	1.000
Non-Hispanic	24 (85.7)	26 (92.9)	17 (89.5)		
Education (N, %)					
Less than High School	1 (3.6)	1 (3.6)	1 (5.3)		
High School certificate	8 (28.6)	9 (32.1)	5 (26.3)		
Some college	14 (50.0)	5 (17.9)	5 (26.3)	0.131	0.301
Bachelor degree	3 (10.7)	8 (28.6)	3 (15.8)		
Master degree	2 (7.1)	2 (7.1)	26.3 (5)		
Professional/Doctorate	0	3 (10.7)	0		

a: Ketamine cases versus Non-ketamine controls

b: Patients exposed to Ketamine who experienced EP (cases) versus patients who did not experience EP (Controls)

**Validation of the Clinician Administered Dissociative State Scale (CADSS):
CADSS has a Cronbach's alpha of 0.82 and reliably discriminated ketamine-
induced EP from other variables**

Difference in Severity of Dissociation by ketamine Administration						
	Total CADSS Score			U	p	
	\bar{x}	sd	Median			
No Ketamine (n = 28)	1.0	1.78	0	328	<0.001	
Ketamine (n= 47)	4.74	5.59	3			
Male (n = 35)	3.4	5.35	1	670.5	0.750	
Female (n = 40)	3.3	4.52	2			
Occurrence of Ketamine-Induced Emergence Phenomena						
	No EP		EP		Fisher's exact p-value	OR (95% CI)
	n	%	n	%		
No Ketamine (n= 28)	26	92.9	2	7.1	0.003	8.821 (1.869, 41.634)
Ketamine (n= 47)	28	59.6	19	40.4		
Male (n= 35)	26	74.3	9	25.7	0.798	0.808 (0.292, 2.231)
Female (n= 40)	28	70	12	30		

Hardy Weinberg Equilibrium

rs3745274 rs1019385 rs1806191
 *p<0.05
 a Major/minor allele, major and minor z
 b Number of samples that are genotyped



Using Logistic Regression to Predict EP Occurrence

Predictor	Wald p-value	OR	90% CI		Model χ^2	Model p value	Nagelkerke's R ²	Prediction
rs3745274	0.313	2.758	0.528	14.398				
Age	0.051	0.932	0.879	0.989				
Duration of Anesthesia	0.021	1.050	1.014	1.087	19.389	0.002	0.477	77.8
Ketamine dose	0.003	1.161	1.069	1.261				
Time to CADSS	0.012	0.939	0.901	0.978				

rs2279343	0.587	0.561	0.310	10.233				
Age	0.040	0.922	0.864	0.984				
Duration of Anesthesia	0.013	1.064	1.021	1.110	23.098	<0.001	0.559	79.5
Ketamine dose	0.002	1.193	1.085	1.312				
Time to CADSS	0.010	0.929	0.886	0.973				

rs1019385	0.668	1.529	0.301	7.776				
Age	0.066	0.943	0.895	0.994				
Duration of Anesthesia	0.026	1.053	1.014	1.094	18.468	0.002	0.458	77.8
Ketamine Dose	0.002	1.142	1.064	1.226				
Time to CADSS	0.019	0.941	0.901	0.982				

rs1806191	0.672	0.685	0.158	2.973				
Age	0.064	0.944	0.897	0.994				
Duration of Anesthesia	0.025	1.052	1.014	1.092	8.458	0.002	0.458	80
Ketamine dose	0.002	1.138	1.063	1.219				
Time to CADSS	0.017	0.942	0.903	0.982				

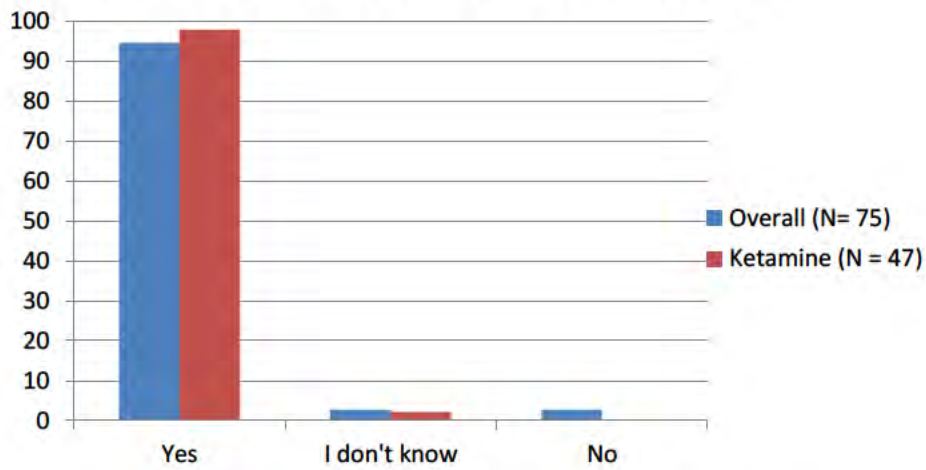
*Using Logistic Regression to Predict EP Occurrence:
Non-genetic variable significantly predicted the occurrence of EP*

	OR	Wald <i>p-value</i>	90% CI	Model χ^2	Model <i>p value</i>	Nagelkerke's R^2	Prediction
Ketamine dose	1.135	0.002	1.063 1.211	18.251	0.001	0.435	76.6
Age	0.952	0.093	0.907 0.999				
Duration of Anesthesia	1.044	0.033	1.010 1.078				
Time to CADSS	0.949	0.020	0.915 0.985				

*Using Linear Regression to Prediction EP Severity:
Non-Genetic variables significantly predicted the severity of EP*

Predictor	B	β	P value	90% CI	F	Model p value	R^2	Adjusted R^2
Ketamine dose	0.048	0.527	0.001	0.026 0.070				
Duration of Anesthesia	0.016	0.457	0.056	0.002 0.030				
Age	-0.025	-0.264	0.057	-0.046 0.004	4.028	0.007	0.277	0.208
Time to CADSS	-0.019	-0.509	0.042	-0.34 -0.004				

Future Acceptance of same anesthetic



Use of ketamine and EP occurrence does not affect satisfaction with care provided: Average satisfaction score 96% satisfaction

Highlights

- CADSS reliably distinguished ketamine-induced EP from other post-anesthetic behavior
- 40.4% occurrence of EP is consistent with literature
- Lack of association between CYP2B6 and EP has been reported in patients with treatment resistant depression and chronic pain
 - However studies were underpowered

Highlights

- Non genetic variables significantly predicted occurrence and severity of EP
 - OR of EP with ketamine dose= 1.135
 - Adjusted R² for EP severity = 20.8%
- EP does not affect patient satisfaction with the anesthetic

Implications for Clinical practice

- Use ketamine when appropriate
- Closely monitor patients
- Titrate dose carefully

Implications for Research

- Feasibility of Approach
- Sample Size estimation

rs2279343
rs1019385
rs1806191

MAF = Minor allele frequency
a = sample size for cases only (match for control to obtain total sample size)
b = Odds ratio of EP occurrence by genotype adjusted for ketamine dose, age, duration of surgery, time from ketamine administration to assessment of dissociation

Implications for Research

- Feasibility of approach
- Sample size estimation

dbSNP ID	MAF	OR	Sample Size ^a	Adjusted OR ^b	Sample Size ^a
rs3745274	0.26	1.125	384	2.7	78
rs2279343	0.28	1.48	390	1.8	138
rs1019385	0.50	1.41	570	1.5	570
rs1806191	0.50	1.25	570	1.5	570

MAF = Minor allele frequency; OR = gene only odds ratio to EP occurrence,
a = sample size for cases only (match for control to obtain total sample size)
b = Odds ratio of EP occurrence by genotype adjusted for ketamine dose, age, duration of surgery, time from ketamine administration to assessment of dissociation

Limitations

- Small Sample Size
- Caucasian, middle aged and well educated
- Other SNPs in CYP2B6 and GRIN2B genes
- Ketamine metabolites

Strengths

- Stringent inclusion and exclusion criteria
- Real live clinical setting
- One observer
- Genotype controls

Conclusion

- Conduction of a well powered pharmacogenetic study is feasible
- Despite concerns by providers, ketamine-induced EP does not seem to affect satisfaction with the anesthetic
- The odds of EP occurrence are 3.52 times higher with every additional 1ml (10mg) of ketamine

Funding

- Fairlawn Scholarship
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- Dr Singla Sudershan, Chief of Community Anesthesia
- Dr Steve Heard, Director of Research, Dep't of Anesthesiology
- Dr Shubjeet Kaur, Chair of Anesthesia
- PACU/SACU Nurses- UMMHC Hahnemann and Memorial Campuses
- Department of Anesthesiology Colleagues
- UMW GSN Faculty and Fellow Students
- My wife, kids, brothers, sisters, nieces and nephews

Questions

DISSEMINATION PLAN

The primary description of this dissertation work has been submitted as a manuscript on April 30th 2016 to *Nursing Research* for review and consideration for publication. A podium presentation “Lack of Association of CYP2B6*6 And GRIN2B Alleles with Ketamine-Induced Emergence Phenomena: A pilot Study” will be presented on August 5th 2016 at the International Society of Nurses in Genetic (ISONG) World Congress, Dublin, Ireland.

APPENDIX

Demographic Sheet

Please do not write your name on this form. This information will allow us to describe all the people who took part in the study.

Study ID _____

Date of birth _____

Age (years) _____

Gender

- Female
- Male
- Transgender

Ethnicity

- Hispanic or Latino
- Not Hispanic or Latino
- Unknown / Not Reported

Race

- Caucasian
- Asian
- Black or African American
- American Indian
- Other

Do you currently smoke?

- Yes
- No

If Yes, How many packs per day _____

Have you EVER smoked?

- Yes
- No

When did you quit?

- Less than 1 month ago
- 1 month to 1 year ago
- 1 year to 5 years ago
- Over 5 years ago

Do you smoke marijuana?

- Yes
- No

Do you use any other recreational drugs?

- Yes
- No

Please list recreational drugs

Do you drink alcohol every day?

- Yes
- No

How much in a typical week?

What is your highest level of education completed?

- Did Not Complete High School
- High School/GED
- Some College
- Bachelor's Degree
- Master's Degree
- Advanced Graduate work or Doctorate

Weight (kilograms)

Height (cm)

BMI

Comments

Data Collection Sheet

Study ID	_____
CADSS time (Copy from CADSS form)	_____
PACU Arrival time	_____
Weight (Kg)	_____
Height (cm)	_____
Ketamine Time	_____
Ketamine (mg)	_____
Ketamine (mg/kg)	_____
Midazolam (mg)	_____
Midazolam (mg/kg)	_____
PACU to CADSS time	_____
Type of Procedure	_____
Time since last dose of Ketamine (mins)	_____
Duration of Anesthesia (mins)	_____
Other Sedatives (mg)	_____
Other Perioperative Medications (Name and Dose)	_____ _____
Anesthesia Complications	_____
Current Medications	_____
Other Co-morbidities	_____
Comments	_____
Duration in PACU (minutes)	_____

Clinician Administered Dissociative States Scale (CADSS)

Subjective Items:

1. Do things seem to be moving in slow motion?
 - 0= Not at all.
 - 1= Mild, things seem slightly slowed down, but not very noticeable.
 - 2= Moderate, things are moving about twice as slow as normally.
 - 3= Severe, things are moving so slowly that they are barely moving.
 - 4= Extreme, things are moving so slowly, I have the perception that everything has come to a stop, as if time is standing still.

2. Do things seem to be unreal to you, as if you are in a dream?
 - 0= Not at all.
 - 1= Mild, things seem a little unreal, but I'm well aware of where I'm at.
 - 2= Moderate, things seem dreamlike, although I know I am awake.
 - 3= Severe, things seem very dreamlike, although I know that I am here, I have the feeling like I might be asleep.
 - 4= Extreme, I feel like nothing is real, like I should pinch myself to wake up, or ask someone if this is a dream.

3. Do you have some experience that separates you from what is happening; for instance, do you feel as if you are in a movie or a play, or as if you are a robot?
 - 0= Not at all.
 - 1= Mild, I feel a little bit separated from what is happening, but I am basically here. 2= Moderate, I feel somewhat separated from what is going on, or I feel as if I am in a movie or a play.
 - 3= Severe, I feel extremely separated from what is going on, or I feel as if I am in a movie or a play.
 - 4= Extreme, I feel as if everyone around me is talking a foreign language, so that I cannot understand what they are saying, or I feel as if I am on the outside looking in, or like I am a robot or a machine.

4. Do you feel as if you are looking at things from outside of your body?
 - 0= Not at all.
 - 1= Mild, I feel somewhat disconnected from myself, but I am basically all together. 2= Moderate, I feel like I am outside of my body, but not looking down upon myself from far above.
 - 3= Severe, I feel like I am twenty feet or more away from my body, looking down from above.
 - 4= Extreme, I feel as if I am hundreds of feet above myself, looking down at myself and everyone else here.

5. Do you feel as if you are watching the situation as an observer or a spectator?

0= Not at all.

1= Mild, I feel slightly detached from what is going on, but I am basically here.

2= Moderate, I feel somewhat removed as an observer or a spectator, but I am definitely in this room.

3= Severe, I feel very much as if I am an observer or a spectator, but I am still here in this room.

4= Extreme, I feel completely removed from what is happening, as if I am not a part of this experience in any way, but totally removed from what is happening, as an observer or a spectator.

6. Do you feel disconnected from your own body?

0= Not at all.

1= Mild, I feel a little bit disconnected from myself, but I am basically all here.

2= Moderate, I feel somewhat detached from my own body, but I am basically all together.

3= Severe, I feel detached from my own body, but not far removed from my body, and I feel as if it is me there.

4= Extreme, I feel like I am completely out of my body, as if I am looking at my own body from a long way off, as if there is another person there.

7. Does your sense of your own body feel changed: for instance, does your own body feel unusually large or unusually small?

0= Not at all.

1= Mild, I have a vague feeling that something about my body has changed, but I can't say exactly what it is.

2= Moderate, I feel like my body has increased or decreased in size slightly, or that it feels somewhat as if it is not my body.

3= Severe, I feel as if my body has increased to twice its normal size, or decreased to twice its normal size, or I very much feel that this is not my body.

4= Extreme, I feel as if my body has swelled up to at least ten times its normal size, or as if it is ten times as small. Or as if my arms have become like toothpicks.

8. Do people seem motionless, dead, or mechanical?

0= Not at all.

1= Mild, people seem a little bit more motionless, dead, or mechanical than would be normal.

2= Moderate, people seem to be at least twice as motionless or mechanical than would be normal.

3= Severe, people seem to be barely moving, or barely alive, or very mechanical.

4= Extreme, it's as if everyone were frozen or completely like machines.

9. Do objects look different than you would expect?

0= Not at all.

1= Mild, things seem slightly different than normal, although it is barely perceptible. 2= Moderate, things are somewhat distorted, but I have no problems recognizing things around me.

3= Severe, things are much more distorted, but I have no problems recognizing things around me.

4= Extreme, like everything is distorted, not real, I feel like I cannot recognize anything, everything is alien or strange.

10. Do colors seem to be diminished in intensity?

0= Not at all.

1= Mild, things seem slightly paler than usual if I think about it.

2= Moderate, colors are somewhat diminished, but still recognizable.

3= Severe, colors are extremely pale, in no way as vivid as they usually are.

4= Extreme, everything is black and white, or all the colors have been washed out.

11. Do you see things as if you were in a tunnel, or looking through a wide angle photographic lens?

0= Not at all.

1= Mild, I feel a little bit like I am looking through a tunnel, or a wide angle lens.

2= Moderate, the periphery of my vision is blacked out, but I still have most of my visual field, or things are somewhat like a wide angle lens.

3= Severe, it seems as if I'm looking through a tunnel, or through a wide angle lens, but I can see everything clearly.

4= Extreme, as if I'm looking through a pair of binoculars backwards, where everything around the periphery is blacked out, and I can see a little point of light at the end of a tunnel, with little tiny people and objects, or I am seeing things as if through a wide lens and things are incredibly expanded.

12. Does this experience seem to take much longer than you would have expected?

0= Not at all.

1= Mild, it seems as if the interview has gone by for at least twice as long as the true elapsed time.

2= Moderate, it seems as if the interview has gone by for at least two hours.

- 3= Severe, it seems as if at least ten hours have gone by since the start of the interview.
- 4= Extreme, it seems as if time is standing still, so that we have been here at this point in time for ever.

13. Do things seem to be happening very quickly, as if there is a lifetime in a moment?

- 0= Not at all.
- 1= Mild, things are happening slightly faster than normal.
- 2= Moderate, things seem to be happening at least twice as fast as normal.
- 3= Severe, things seem to be happening at least 10 times faster than normal.
- 4= Extreme, as if this whole experience has happened at once, or as if there is a lifetime in a moment.

14. Do things happen that you later cannot account for?

- 0= Not at all.
- 1= Mild, there may have been things which happened which now I can't account for, but nothing pronounced.
- 2= Moderate, at least once there were things which happened which now I can't account for.
- 3= Severe, at least twice I have lost several minutes of time, so that now there are things I cannot account for.
- 4= Extreme, large pieces of time are missing, of ten minutes or more, so that I am confused about what has happened.

15. Do you space out, or in some other way lose track of what was going on?

- 0= Not at all.
- 1= Mild, I have had some episodes of losing track of what is going on, but I have followed everything for the most part.
- 2= Moderate, I have lost at least a minute of time, or have completely lost track of what is going on now.
- 3= Severe, I have lost several segments of time of one minute or more.
- 4= Extreme, I have lost large segments of time of at least 15 minutes or more.

16. Do sounds almost disappear or become much stronger than you would have expected?

- 0= Not at all.
- 1= Mild, things are a little quieter than normal, or a little louder than normal, but it is not very noticeable.

- 2= Moderate, things have become about twice as soft as normal, or twice as loud as normal.
- 3= Severe, things have become very quiet, as if everyone is whispering, or things have become very loud (although not deafening).
- 4= Extreme, things have become completely silent, or sounds are so loud that it is deafening, and I feel as if I am going to break my eardrums.

17. Do things seem to be very real, as if there is a special sense of clarity?

- 0= Not at all.
- 1= Mild, things seem to be a little bit more real than normal.
- 2= Moderate, things seem to be more real than normal.
- 3= Severe, things seem to be very real or have a special sense of clarity.
- 4= Extreme, things seem to have an incredible sense of realness or clarity.

18. Does it seem as if you are looking at the world through a fog, so that people and objects appear far away or unclear?

- 0= Not at all.
- 1= Mild, things seem somewhat foggy and unclear, or I do have the feeling that things are far away, but there is not a major effect on how I perceive things around me.
- 2= Moderate, things seem very foggy and unclear, or things seem like they are far away, but I can identify the interviewer and objects in the room easily.
- 3= Severe, I can barely see things around me, such as the interviewer and the objects in the room.
- 4= Extreme, I cannot make out anything around me.

19. Do colors seem much brighter than you would have expected?

- 0= Not at all.
- 1= Mild, colors seem a little bit brighter than normal, but not more than twice as bright.
- 2= Moderate, colors seem brighter, about twice as bright as normal.
- 3= Severe, colors seem very bright, at least five times as bright as normal.
- 4= Extreme, colors seem extremely bright, almost fluorescent, at least 10 times as bright as normal.

20. Do you feel confused about who you really are?

- 0= Not at all.
- 1= Mild, I feel a little bit confused about who I am.
- 2= Moderate, I feel confused about who I am, but I basically know who I am.
- 3= Severe, I feel very confused about who I am, and at times I wonder if I am a person, or if I am many people.
- 4= Extreme, I feel as if there were two or more sides to myself.

21. Do you feel like there are different parts of yourself which do not fit together?
- 0= Not at all.
 - 1= mild, I feel like there are different sides of myself, but they're basically part of myself.
 - 2= Moderate, I feel like I have different parts which don't quite fit together.
 - 3= Severe, there are two or more sides to myself which have unique characteristics.
 - 4= Extreme, I have two or more parts to myself with unique personality characteristics.
22. Do you have gaps in your memory?
- 0= Not at all.
 - 1= Mild, there are some recent things which I cannot remember.
 - 2= Moderate, there have been a few gaps in my memory which lasted a few minutes.
 - 3= Severe, there have been large gaps in my memory which lasted for more than a few minutes.
 - 4= Extreme, I cannot piece together what is happening from one moment to the next due to large gaps in my memory.
23. Do you feel like you have more than one identity?
- 0= Not at all.
 - 1= Mild, I feel like there is more to me than my personality, but it's basically part of my identity.
 - 2= Moderate, I feel like I have more than one personality, but the personalities are not really distinct.
 - 3= Severe, I have two or more personalities, although they are not fully developed as distinct entities.
 - 4= Extreme, I have two or more personalities which are distinct and have their own names and other unique characteristics.

Supplemental Question:

29. If presented with a choice in the future, will you accept the same anesthetic?
- _____ NO
 - _____ I don't know
 - _____ Yes
30. On a scale of 1 to 100 how satisfied were you with the anesthetic? _____
- 1= not satisfied at all.
 - 100= extremely satisfied

Observer Items:

24. Did the subject seem eery or strange, or in some other way give you an uncomfortable feeling?

0= Not at all.

1= Mild, the subject seemed a little bit eery or strange.

2= Moderate, the subject seemed somewhat eery and strange and gave me an uncomfortable feeling.

3= Severe, the subject seemed very eery and strange and I was very uncomfortable sitting with this person.

4= Extreme, the subject seemed very eery and strange and I was extremely uncomfortable sitting with this person.

25. Did the subject blank out or space out, or in some other way appear to have lost track of what was going on?

0= Not at all.

1= Mild, the subject seemed a little bit out of it, but not very noticeable.

2= Moderate, the subject on more than one occasion spaced out, blanked out, or in some other way showed evidence of having lost some time.

3= Severe, the subject at least twice blanked out, spaced out, or in some other way showed evidence of having lost some time.

4= Extreme, the subject was continuously blanking out and losing track of what was going on.

26. Did the subject appear to be separated or detached from what is going on, as if not a part of the experience or not responding in a way that you would expect?

0= Not at all.

1= Mild, somewhat detached from what was going on.

2= Moderate, as if the subject is there but not there at the same time.

3= Severe, so that the subject definitely appears to be detached from his or her surroundings.

4= Extreme, appearing totally numbed out and not a part of what is going on around him or her.

27. Has the subject had to be put back on track, or grounded in the here and now, during or soon after the experience?

0= Not at all.

1= Mild, the subject seemed a little bit out of it, but followed what we were doing and answered all of the questions appropriately.

2= Moderate, at least once I had to tell the subject what we were doing or had to repeat a question.

- 3= Severe, at least once I had to tell the subject where he was or who he was, or two times or more I had to tell the subject what we were doing, or two times or more I had to repeat questions.
- 4= Extreme, I was continuously reminding the subject of what we were doing, or had to tell the subject more than once where he was or who he was, or was continuously repeating questions.

28. Did it seem as if there were different aspects of the subject's personality which did not fit together?

- 0= Not at all, the subject's personality was very clear.
- 1= Mild, sometimes the subject would do or say something which didn't seem to fit in with their personality.
- 2= Moderate, the subject sometimes talked or acted in different ways.
- 3= Severe, there seemed to be different parts of the subject's personality which did not fit in with the whole.
- 4= Extreme, the subject specifically referred to two different aspects of their personality and showed some evidence of more than one unique personality.

Study Flyer

**PHARMACOGENETICS OF KETAMINE-
INDUCED EMERGENCE PHENOMENA:
A PILOT STUDY**

Are you an adult having anorectal, hand or foot surgery at UMass Memorial, Hahnemann, or University Campus?

You may be asked to be in a research study to better understand why people who get ketamine (a drug for anesthesia) during surgery may have different responses.

The researcher will be present on the day of your surgery to discuss the study with you, answer all your questions, and ask for your consent to participate.

Thank You

PRINCIPAL INVESTIGATOR
Edwin N. Aroke, MSN, CRNA,
PhD Candidate, Graduate School of Nursing
UMASS, Worcester
Tel: (617) 818 0585

Remember

If you decide to take part in this study, you do so as a VOLUNTEER.

This means YOU decide.

Your decision will not affect how you are treated.

You can change your mind and leave a research study at any time without affecting how you are treated.

PRINCIPAL INVESTIGATOR

Edwin N. Aroke, MSN, CRNA

PhD Candidate

Phone: 617-818-0685

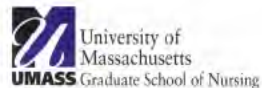
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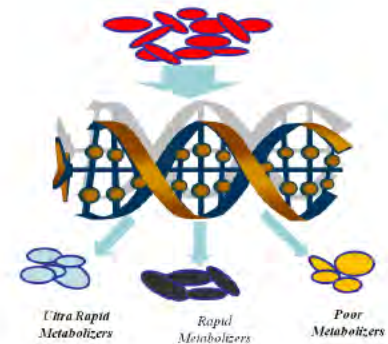


IRB ID# H00007484

GRADUATE SCHOOL OF
NURSING

Pharmacogenetics of Ketamine-Induced Emergence Phenomena

A Pilot Study



**INFORMATION FOR
RESEARCH SUBJECTS**



One of the medicines used when people have surgery is called **ketamine**. Not everyone who gets this medicine responds the same way. When some people get this medicine they have different kinds of dreams, an out of body experience, or a general sense that they are in a different space. These effects only last 2-3 hours and go away on their own. We think that the reason not everyone gets the same effects from ketamine is because of a difference in their genes. We want to find out if the type of genes people have is related to the type of response they get from this medicine.

Joining this study is entirely voluntary.

FREQUENTLY ASKED QUESTIONS

Why are you being invited to take part in a research study?

You are being asked to take part in this study because you are having surgery and will receive anesthesia

What should you know about a research study?

Your participation is entirely voluntary.

You do not have to be in this research study. If you join the study, you can stop or leave at any time with no changes in the quality of the health care you receive.

You will be told about any new information or changes in the study that could affect you.

You can ask all the questions you want before deciding if you want to be in this study.

How long will the research last?

We expect that you will be in this study for less than 2 hours.

What are the risks of being in this study?

The minimal risks of being in this study include becoming upset from talking about the experience, breach of confidentiality, and genetic testing.

What happens if I say yes, I want to be in this research?

Before your surgery, you will fill out a short form about yourself – things like age, education, race, use of alcohol and if you smoke.

After your surgery we will ask you questions about how you feel and 2 teaspoons of your blood will be taken.

The blood will be used for genetic testing and some will be stored.

Will being in this study help me in any way?

No, the results of the study may help others in the future, but there is no direct benefit to you