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Pharmacogenetics of Ketamine-Induced Emergence Phenomena: A Pilot Study

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Abstract

Background—Up to 55% of patients administered ketamine, experience an emergence phenomena (EP) that closely mimics schizophrenia and increases their risk of injury, but to date no studies have investigated genetic association of ketamine-induced EP in healthy patients.

Objectives—To investigate the feasibility and sample sizes required to explore the relationship between *CYP2B6**6 and *GRIN2B* single nucleotide polymorphisms (SNPs) and ketamine-induced emergence phenomena (EP).

Methods—This cross-sectional pharmacogenetic candidate gene pilot study recruited 75 patients having minor elective outpatient surgeries. EP was measured with the Clinician Administered Dissociative State Scale (CADSS). Genetic association of *CYP2B6**6 and *GRIN2B* (rs1019385 & rs1806191) SNPs and ketamine induced EP occurrence and severity were tested using logistic and linear regression.

Results—Forty-seven patients (63%) received ketamine and were genotyped, and forty percent of them experienced EP. Occurrence and severity of EP were not associated with *CYP2B6**6 or *GRIN2B* ($p > 0.1$). Exploratory analysis of non-genotype models containing age, ketamine dose, duration of anesthesia, and time since ketamine administration significantly predicted EP occurrence ($p = 0.001$) and severity ($p = 0.007$). This pilot study demonstrates feasibility for implementing a pharmacogenetic study in a clinical setting, and we estimate that between 380 and 570 cases will be needed to adequately power future genetic association studies.

Discussion—Younger age, higher dose, and longer duration of anesthesia significantly predicted EP occurrence and severity among our pilot sample. While the small sample size limited our ability to demonstrate significant genotype differences, we generated effect sizes, sample size estimates and non-genetic covariates information in order to support future pharmacogenetic study design for evaluating this adverse event.

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Keywords

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Ketamine-induced emergence phenomena (EP) occur in up to 55 percent of patients treated with ketamine (Kumar, Bajaj, Sarkar, & Grover, 1992; Strayer & Nelson, 2008). It is characterized by euphoria, vivid dreams, illusions, delirium, and hallucinations (Aroni et al., 2009; Pomarol-Clotet et al., 2006; Powers III, Gancsos, Finn, Morgan, & Corlett, 2015; Xu & Lipsky, 2014). EP has equally been described as floating sensation with distortions in body images and objects, which correlate with positive and negative psychotic symptoms of schizophrenia (Pomarol-Clotet et al., 2006; Xu et al., 2015). Single-photon emission tomographic studies revealed that ketamine produces negative psychotic symptoms by direct inhibition of the N-methyl-D-aspartate receptor (NMDAR) (Stone et al., 2008), while positive symptoms correlated with blood ketamine levels and glutamate release from the anterior cingulate cortex (Stone et al., 2012; Stone et al., 2008). Thus, EP may be related to NMDAR function, and rate of ketamine metabolism. Beside increasing healthcare cost (Franco, Litaker, Locala, & Bronson, 2001), EP increases patients' risk of injury, length of hospital stay, requires more nursing care, and contributes to patient's lower satisfaction with care (Hudek, 2009; O'Malley, 2014; Stoelting & Hillier, 2006). While several medications such as midazolam (Sener, Eken, Schultz, Serinken & Ozsarac, 2011) and propofol (Andolfatto & Willman, 2011) have been used to manage EP, to date there is no definitive treatment or predictive tools to identify patients who will develop EP. As a result, most clinicians avoid using ketamine despite its diverse clinical profile.

Clinical uses of ketamine include induction of anesthesia, procedural sedation, acute and chronic pain management, treatment of depression (Aroni, Iacovidou, Dontas, Pourzitaki, & Xanthos, 2009; Mion & Villeveille, 2013; Zarate, Brutsche, Ibrahim, et al., 2012; Zarate, Brutsche, Laje, et al., 2012), and modulation of inflammation (D'Alonzo et al., 2011; Dale, Somogyi, Li, Sullivan, & Shavit, 2012). Ketamine is essential 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).

Ketamine is a unique anesthetic that exerts most of its effects by non-competitive antagonism of the NMDAR. Unlike other anesthetics such as propofol, patients under ketamine-induced loss of consciousness maintain spontaneous respiration and protective airway reflexes. In addition, it has sympathomimetic (increase heart rate, blood pressure and cardiac output) and bronchodilatory effects, which makes it the preferred anesthetic in patients with hemodynamic instability such as hypovolemia, cardiogenic shock and constrictive cardiac disorders (Aroni et al., 2009) and severe asthma (Lau & Zed, 2001). 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), and endoscopy and radiology suites (Mason et al., 2002). In addition,

ketamine has been widely used to provide sedation and analgesia in burn units and emergency departments. The major advantage of ketamine in these units lies not only in its analgesic effects, but in the fact that it can be administered intramuscularly, orally, rectally, and transnasally. This is desirable in uncooperative children and patients with extensive burns, who may have limited intravenous (IV) access.

While the causes of adverse drug reactions are multi-factorial, genetic predisposition has been estimated to cause about 50 percent of severe adverse drug reactions and account for up to 95 percent of drug response variability (Chidambaran, Ngamprasertwong, Vinks, & Sadhasivam, 2012). Ketamine exerts most of its clinical effects by non-competitively binding to the NR2 subunit of the NMDAR and blocking the excitatory effects of glutamate (its principal neurotransmitter). Genetic polymorphism of the NMDAR gene (*GRIN2*) has been associated with NMDAR hypo-function, psychotic symptoms, and memory disorders (Frohlich & Van Horn, 2014; Li & He, 2007; Xu & Lipsky, 2014). Two single nucleotide polymorphisms (SNPs) (T200G [rs1019385], 4197T/C and rs1806194 in the *GRIN2B* gene have been linked to schizophrenia in susceptible individuals (Demontis et al., 2011). Thus, ketamine-induced EP may be related to genetic alternations in the NMDAR- NR2B subunit.

Additionally, ketamine is metabolized primarily by enzymes coded by *CYP2B6* gene (Hijazi & Boulieu, 2002; Yanagihara et al., 2001). Besides disease conditions such as infection and inflammation (Cheng & Morgan; Renton, 2005) SNPs in drug metabolizing enzymes can alter drug metabolism (Zanger & Klein, 2013). *CYP2B6**6 is a loss of function allele that has two non-synonymous SNPs, Gln172His (rs3745274, c.516G>T) and Lys262Arg (rs2279343, 785A>G), in exons 4 and 5 respectively. *CYP2B6**6 allele significantly affects ketamine metabolism (Li et al., 2013) and has been associated with increased drowsiness in patients with chronic pain (Li et al., 2015). However, to date, no study has investigated the genetic associations between *CYP2B6* and *GRIN2B* alleles and EP in relatively healthy patients.

The primary objective of this pilot study was to explore genetic association between *CYP2B6*, and *GRIN2B* SNPs and the incidence and severity of ketamine-induced EP in relatively healthy patients. Secondary aims included determination of the effect size of EP and effect of EP on patient satisfaction.

Method

Participants and Settings

Participants were recruited from an ambulatory surgical center and a community hospital, which are affiliated with a large academic medical center in central Massachusetts. A patient was eligible to participate if he/she was relatively healthy (American Society of Anesthesiology physical status classes (ASA) I & II); 18 years; undergoing minor orthopedic hand foot, or anorectal surgery under sedation; able to read, write and understand English; and able to give informed written consent. ASA 1 patients are considered 'normal healthy' patients, while ASA II are those with mild systemic disease but without substantive functional limitations. Examples include (but are not limited to): current smoker, social alcohol drinker, pregnancy, obesity (30 < BMI < 40), well-controlled DM/HTN, mild lung

disease. Patients with liver diseases that can affect drug metabolism or elevated liver enzymes are classified as ASA III or above, and thus not included in this study. Patients were excluded if they had a diagnosis of depression, schizophrenia, or bipolar disorder. Equally excluded were patients taking medications known to induce or inhibit the activity of CYP2B6 enzymes such as rifampin, dilantin, and phenobarbital. A total of 135 patients were approached to participate, 88 were enrolled, and 75 completed the study (figure 1). Thirteen participants were either dropped from the study because of conversion to general anesthesia or participants' unwillingness to continue to the study.

Study Procedures

The study was approved by the University of Massachusetts Medical School Institutional Review Board (IRB). An IRB waiver was obtained to access the operating room schedule to screen for potential participants. During the preoperative period, the admitting nurse introduced the study to the patients and those interested then were introduced to the principal investigator (PI) who discussed the study, answered all questions and obtained informed written consent prior to the scheduled surgery and before the administration of any sedatives or hypnotics. After providing written informed consent, participants completed a demographic questionnaire. Patients underwent their scheduled surgery and all decisions regarding type and dose of anesthetic were determined by the anesthesia provider. Those given intra-operative ketamine received a dose of 0.5mg/kg as is standard for these procedures. The specific dose and use of other perioperative medications was at the discretion of the anesthesia provider. Within one hour of arrival to the post-anesthetic care unit (PACU), the Clinician Administered Dissociative State Scale (CADSS) was administered by the PI. Information about perioperative medications was abstracted from the medical record, and a blood sample was obtained from the existing IV line or by direct venous puncture. Blood was stored at stored at -80 degree Celsius until DNA extraction.

Instruments and Phenotyping

All data were entered directly into REDCap (Research Electronic Data Capture), a secure web-based application for managing online surveys and databases for research. Demographic data included age, gender, ethnicity, race, education, smoking status, and alcohol consumption. The CADSS was used to evaluate ketamine induced emergence phenomena. CADSS scores range from 0 to 112, with scores 4 indicating positive dissociation, and higher scores indicating increase severity of dissociation (Bremner, 2014). CADSS has an internal consistency Cronbach's alpha of 0.94 (Bremner et al., 1998). To assess the incidence of EP it was coded as a binary variable: a total CADSS score of less than 4 was considered to be EP negative, while a total CADSS score of 4 was considered to be EP positive. The severity of EP was a continuous variable with higher CADSS scores indicating more EP.

We also recorded dose of ketamine administered, other perioperative medications given, and overall satisfaction with anesthesia care. As is common practice in these facilities, 98 percent of participants received pre-operative midazolam. Prior to discharge from the post-anesthesia care unit (PACU) each participants was asked to rate their satisfaction with the anesthetics on a scale of 0 to 100, with 0 indicating total dissatisfaction and 100 indicating

total satisfaction. In addition, participants were asked to indicate their willingness to accept the same anesthetics in the future as “yes,” “I don’t know” or “no.”

Genetic Data

Gene/SNP Selection—Gene/SNP selection for analysis was driven by the literature. Ketamine is metabolized primarily by enzymes coded by the *CYP2B6* with minor contributions from the *CYP2C9* and *CYP3A4* (Hijazi & Boulieu, 2002; Yanagihara et al., 2001). *CYP2B6* has many functional SNPs that alter drug action (Zanger & 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 (Zanger & Klein, 2013). *In vitro* studies show that *CYP2B6**6 allele significantly alters ketamine metabolism (Li et al., 2013). Recently, among patients with chronic pain the *CYP2B6**6 allele has been associated with decreased ketamine clearance and increased drowsiness (Li et al., 2015).

Ketamine is primarily an N-methyl-D-aspartate receptor (NMDAR) antagonist. It binds to the NR2 subunit of the NMDA receptor and blocks the action of glutamate. Functional MRI studies have shown that ketamine induces the release of glutamate in manner similar to that seen in NMDA receptor hypofunction (Frohlich & Van Horn, 2014). NMDAR hypofunction and glutamate hyperactivity are the molecular bases of schizophrenia (Javitt, 2010). In addition, genetic polymorphisms in the glutaminergic pathway have been associated with NMDAR hypofunction and symptoms of schizophrenia, learning, and memory disorders (Frohlich & Van Horn, 2014; Li & He, 2007; Xu & Lipsky, 2014). Two SNPs (T200G (rs1019385), 4197T/C and rs1806194) in the NMDAR gene (*GRIN2B*) have been linked to schizophrenia in susceptible individuals (Demontis et al., 2011; Li & He, 2007). Thus, ketamine-induced EP may be related to NMDAR- NR2B subunit function. Two candidate SNPs from the *CYP2B6**6 and *GRIN2B* genes were selected for analysis.

Blood collection and genotyping—Of the 47 participants who received ketamine, venous blood was collected from 45 participants into ethylenediaminetetraacetate (EDTA) vacutainer tubes and stored at –80 degree Celsius until DNA extraction. Genomic DNA was extracted from the blood using the QIAgen Protocol for DNA purification from blood (QIAamp DNA Blood Mini Kit, Cat. No 51104). *CYP2B6* SNPs (rs3745274, and rs2279343) and *GRIN2B* SNPs (rs1019385 and rs1806191) were genotyped using standard Taqman Genotyping Assays (Applied Biosystems). All samples were run against control standards (two non-template controls and two known genotype controls—one male and one female from the CEPH (Center d’Étude du Polymorphisme Humain) pedigree sample. As an additional control, 2 study samples were randomly selected and re-genotyped for each of the SNPs.

DNA extraction was performed at the University of Massachusetts Medical School Disease Conquering biorepository, while genotyping was performed at the Duke University Molecular Physiology Institute. Purity and quantification were assessed by measuring the optical density (OD) at 260nm. Laboratory personnel doing the genotyping were blinded to the EP status.

Statistical Analyses

Genotype frequencies of all SNPs were tested for Hardy-Weinberg equilibrium (HWE) by chi square test. We performed statistical analyses using SPSS 22 (Statistical Package for the Social Science [SPSS], 2013). Demographic characteristics were summarized using frequencies and percentages (gender, race, ethnicity, and level of education), and means and standard deviations (age and body mass index). Differences in demographic variables between ketamine versus non-ketamine, and EP positive versus EP negative cases were tested using Fisher's exact, student t, and Mann-Whitney's tests.

Among ketamine recipients, Fisher's exact test was used to evaluate associations between EP occurrence (0, 1) and each SNP using the dominant genetic model. In the dominant model, we tested the effect of having at least one copy of the risk alleles in the SNP on the occurrence of EP. For instance, for the *CYP 2B6* G>T SNP, participants with risk allele genotypes (G/T and T/T) were combined and compared against homozygous wild-type (G/G) participants for EP occurrence. Logistic regression modeling was used to determine the main effect of each genotype on EP occurrence, then adjusting for different variables: age, BMI, gender, ethnicity, level of education, ketamine dose, midazolam dose, propofol dose, duration of surgery, and time from ketamine administration to assessment of dissociation. Non-statistically significant predictors were excluded from the model, while significant covariates (age, ketamine dose, duration of anesthesia and time from ketamine administration to assessment of dissociation) were used to evaluate the relationship between genotype and EP occurrence. Odd ratios (OR) and 90% confidence intervals for risk prediction were estimated from the logistic regression model. Severity of EP was predicted using linear regression modeling adjusting for the same significant covariates. The total CADSS score (severity of EP) was square root transformed to account for right skewness, and the residuals were normally distributed. Collinearity of covariates was tested and all covariates in the model had a variance inflation factor (VIF) of less than 4.

The additive genetic model [that test effect of each risk allele in the SNP in a linear fashion, such that having 1 risk allele (G/T) has a greater effect than having zero risk alleles (G/G), and having 2 risk alleles (T/T) has a greater effect than having only 1 (G/T)] was not tested because of low cell count. Potential interaction with midazolam and propofol were not investigated because 98 percent of patients received both midazolam and propofol. Given the small sample size limiting power, p values of less than 0.1 were considered statistically significant as recommended for pilot studies (Cohen, 1994).

Results

The total sample included 35 males and 40 females with a mean age of 50.5 (SD = 13.9) years. Demographic characteristics are reported in Table 1. Of the 75 participants, 47 received ketamine. The average dose of intravenous ketamine administered was 0.54 (SD = 0.19) mg/kg. There were no statistically significant differences between those who received ketamine and those who did not in terms of gender, race, ethnicity, and educational level ($p > 0.1$). Of the 47 participants who received ketamine, 19 (40.4%) experienced EP (cases), while 28 (59.6%) did not (controls). Among those who received ketamine, there was no

statistically significant difference between age, BMI, gender, race, ethnicity and educational level, and the occurrence of EP ($p > 0.1$).

Clinician Administered Dissociative State Scale (CADSS)

For our population, CADSS has an internal consistency reliability coefficient Cronbach's alpha of 0.82. As expected, the average total CADSS score of participants who did not received ketamine was lower (1.0 (SD = 1.78)), than those of participants who received ketamine (4.74 (SD = 5.59)). This difference was statistically significant (Mann-Whitney $U = 328.0$, $p < 0.001$). The odds of developing EP were 8.8 times higher in patients exposed to ketamine compared to those who did not (95% CI, 1.869, and 41.634). There is a statistically significant relationship between ketamine administration and EP occurrence ($p = 0.003$, Fisher's exact test). This relationship was still significant after controlling for age, duration of surgery, and time from ketamine administration to assessment of EP ($p = 0.002$). The odds of EP occurring increases by 1.135 for every 1 mg increase in the dose of ketamine, 90% CI (1.063, 1.211). There was no statistically significant relationship between BMI and total CADSS score ($r = -0.129$, $p = 0.271$). The total CADSS score did not differ significantly between males and females ($U = 670.5$, $p = 0.750$). Performance on CADSS (0, 1) was not related to BMI or gender ($p = 0.798$, Fisher's exact test).

Candidate Gene Analysis for EP

As summarized in Table 2 samples were successfully genotyped for *CYP 2B6**6(rs3745274 & rs2279343) and two *GRIN2B* (rs1019385 & rs1806191) alleles. All SNPs were in Hardy-Weinberg equilibrium (HWE) in both EP positive (cases) and control groups, except rs1019385 in the control group (MAF 0.46, $p = 0.021$). As such, we re-ran the analysis using Caucasian-only participants and the difference between the observed and expected MAF was still significant ($p = 0.04$). With a 96 percent successful call rate of genotyping, this deviation from HWE may be related to the small cell count. A meta-analysis found that deviation from HWE occurs in about 10 percent case-control genetic association studies but does not postulate dismissal of the findings of the association studies (Trikalinos, Salanti, Khoury, & Ioannidis, 2006). No significant difference was observed in the genotype distribution of the four SNPs between cases and controls. Fisher's exact probability testing of relationship between EP occurrence and genotype (in dominant genetic model) was not statistically significant in all SNPs: rs3745274, rs2279343, rs1019385 & rs1806191 ($p = 1.000$, 0.755, 0.711 and 0.743 respectively).

Assessment of Model Fit

Model fit analyses were completed to determine the non-genetic covariates. Age, ketamine dose, duration of anesthesia, and time from ketamine administration to assessment for EP produced the best fit for predicting EP occurrence by genotype (Table 3). Other potential factors affecting EP such as liver function and concurrent patient use of CYP inducers and inhibitors were controlled for in subject exclusion criteria. All models including the SNPs (rs3745274, rs2279343, rs1019385 & rs1806191) could reliably distinguish between EP cases and controls. Nagelkerke's R^2 indicated moderately strong relationship between prediction and grouping, with overall successful prediction rate of over 77 percent. Age, duration of anesthesia, ketamine dose, and time since ketamine administration made a

significant contribution to the prediction ($p < 0.1$), while genotype was not a significant predictor of EP status ($P > 0.1$). When genotype was removed from the model, all non-genotype predictors (ketamine dose, age, duration of anesthesia, and time since ketamine administration) still made statistically significant contributions to the model ($p < 0.1$). There was no statistically significant interaction between ketamine dose and duration of anesthesia ($p = 0.976$), ketamine dose and time since ketamine administration ($p = 0.810$), and duration of anesthesia and time since ketamine administration ($p = 0.860$).

Total CADSS scores ranged from 0 to 22 (mean 4.74, SD = 5.59). There was no statistically significant relationship between EP severity and genotype (dominant model). A linear regression was developed to predict severity of EP based on age, duration of anesthesia, ketamine dose and time since ketamine administration. A significant regression equation was found ($F(4, 42) = 4.028$, $p = 0.007$), with an adjusted R^2 of 21 percent. Age, dose of ketamine, duration of anesthesia and time since ketamine administration made significant contributions to the model (Table 4).

Exploring EP and Satisfaction with Care

Overall, the participants reported over 96 percent satisfaction with the anesthetics. There was no difference in satisfaction between those who received ketamine and those who did not ($t(73) = -0.922$, $p = 0.360$). In addition, seventy-one participants (94.7%) responded “yes,” 2 (2.7%) responded “I don’t know” and 2 (2.7%) responded “no” when asked about their willingness to accept the same anesthetic in the future. Among patients who received ketamine, 97.9% (46) were willing to accept the same anesthetics in the future. Interestingly, EP occurrence did not affect satisfaction with anesthesia care (Mann-Whitney $U = 240$, $p = 0.423$) or willingness to accept the same anesthetics ($p = 0.404$, Fisher’s exact test).

Discussion

To our knowledge, this is the first study to examine the relationship between ketamine induced emergence phenomena and genetic polymorphisms in the pharmacokinetic (CYP2B6) and pharmacodynamic (GRIN2B) pathways of ketamine in relatively healthy patients (ASA I and II).

Ketamine induced emergence phenomena (EP) occurred in 40.4 percent of the patients who received ketamine, consistent with the range reported in the literature (Kumar, Bajaj, Sarkar & Grover, 1992; Strayer & Nelson, 2008). Our small sample of patients experiencing EP ($n = 19$) likely limited our ability to demonstrate a significant association between ketamine induced EP occurrence and *CYP2B6* (rs3745274 and rs2279343) and *GRIN2B* (rs1019385 and rs1806191) SNPs. Previous studies reported lack of association between *CYP2B6* SNPs, and EP in patients with treatment resistant depression (Zarate, Brutsche, Laje, et al., 2012), and chronic pain (Li et al., 2015). However, based on our sample size estimates these studies were underpowered. In addition to low power, one potential reason for lack of association maybe the pathophysiologic effect of diseases such as obesity, inflammation, infection, and diabetes on the phenotypic expression of *CYP2B6* gene. These disease conditions affect CYP expression and activity, thus may alter drug metabolism and disposition (Cheng & Morgan, 2001; Renton, 2005). The influence of infection and inflammation is of particular

interest because many patients in this study underwent anorectal surgery for excision of hemorrhoids (a chronic inflammatory disease with risk for infection). Future genetic studies of the metabolism of ketamine or ketamine induced EP should consider assessing the impact of inflammatory markers, diabetic control (glycosylated hemoglobin), and obesity.

Non-genetic variables (younger age, ketamine dose, duration of anesthesia and time from ketamine administration to assessment of dissociation) appear to predict the occurrence and severity of ketamine induced EP. It is traditionally believed that EP is more common in females, in patients over the age of 16, in short operative procedures, in those receiving large doses and with rapid administration (Frohlich & Van Horn, 2014; Strayer & Nelson, 2008). Our logistic model correctly predicted the occurrence of EP 77 percent of the time, and accounted for about 44 percent of its variance. Some surprising finding emerged from this model: gender was not a significant predictor of EP, and among adults, EP occurrence decreased with age. We speculate that this observation may be due to the fact that for procedural sedation, ketamine is typically administered at the start of surgery (just prior to injection of local anesthesia). Thus, while the dose is titrated to effect (amount needed to tolerate the noxious stimuli) it is not related to the duration of the procedure. Despite the fact that the average dose of ketamine administered was 0.54mg/kg, (which is a “low” or “sub-anesthetic” dose), ketamine dose remained a significant predictor of EP even after adjusting for BMI, age, duration of procedure, and time from ketamine administration to assessment of dissociation. This may have direct clinical implications because, for every 1mg of ketamine administered, the odds of developing EP increased by a factor of 1.135, even with lower baseline doses. Adequately powered future studies are needed to support whether anesthesia providers should change current practice. However, our preliminary findings suggest that administration of just 1ml (10mg) of ketamine increased the odds of developing EP three and half (3.52) times.

EP occurrence did not affect patient reported satisfaction with the anesthesia care. This observation is contradictory to previous studies (Strayer & Nelson, 2008), and prevailing clinical belief. This may be due to the fact that hallucination and delirium have been used as proxies for dis-satisfaction with care. Anecdotal evidence gathered during the assessment of dissociation revealed that most patients enjoy the dissociative state; however the nurses “dislike” patients experiencing EP. We speculate that dissatisfaction with EP may reflect “provider’s dissatisfaction” with patients’ reaction, and not patients’ dissatisfaction with the experience or care. In a future study, it would be interesting to compare patients’ and nurses’ perceptions of ketamine induced EP. Another plausible explanation of the lack of dissatisfaction could be the co-administration of midazolam and propofol. Previous studies have reported decrease incidence of EP with midazolam (Sener, Eken, Schultz, Serinken, & Ozsarac, 2011) and propofol (Andolfatto & Willman, 2011) administration. However, it is possible that co-administration of these medications with ketamine may convert the dissociative symptoms from frightful unpleasant hallucinations and delusions to pleasant dreams. Future studies could investigate the symptomology of EP from ketamine alone versus ketamine and propofol/midazolam co-administration.

This pilot study provides preliminary data to inform a fully powered pharmacogenetic study investigating ketamine-induced EP. Using Quantos (Gauderman, 2002) we have estimated

sample size requirements for future genetic association studies based on our observed minor allele frequencies (MAF), genotype effect sizes, assuming a dominant genetic model (Table 5). For instance, a genetic association study of EP occurrence by CYP 2B6*6 allele using the dominant genetic model will require 390 cases and 390 controls to achieve a power of 80 percent (Gauderman, 2002). Adequate sample size is one of the most important requirements of a quality pharmacogenetic association study. Besides effect size, the sample size of a pharmacogenetic study must take into account the MAF, genetic model of inheritance (dominant, recessive, co-dominant, additive, or multiplicative), and the number of subgroups to be analyzed. Generally, at the same relative risk and significance level, SNPs with larger MAF will require lower sample size than those with smaller MAF, assuming the same model of inheritance. Similarly, SNPs inherited by dominant model will require smaller sample sizes compare those inherited by recessive or additive model (assuming the same phenotype prevalence, MAF, complete linkage disequilibrium and significance level). Given the complexity of sample size determination in genetic association studies, software such as Quantos (Gauderman, 2002) has been developed. Nurse scientist carrying out genetics or pharmacogenetics association studies are encouraged to consults biostatisticians trained in genetic analyses or use one of these software.

Lesson Learned From this Pilot Study

Lessons learned from this pilot study may help the completion of a fully powered study. As expected, cost and infrastructure are major barriers to completing any pharmacogenetic study. Laboratory space, equipment, storage, and sample processing may be at a premium for many investigators—even if the PI is at a Clinical Translational Science Award (CTSA)-supported campus.

The first step in designing a pharmacogenetic association study is to clearly and accurately define the phenotype of interest (an adverse drug reaction, a desired effect, side effect etc.). Carefully defining the phenotype will improve internal validity, reduce confounding, and improve power to detect association. For this study, while our phenotype, EP, has been described extensively in the literature, no instrument has been developed specifically to measure EP. As a result, proxies such as patients' dissatisfaction, dreams, and instruments used to assess delirium have been used to measure EP. The CADSS was developed to measure dissociative states in PTSD, but has been used in studies of ketamine induced EP. Prior to data analysis, we verified the ability of CADSS to distinguish ketamine induced EP from other post-anesthetics behavior (on average patients who received ketamine scored higher on CADSS than non-ketamine recipients). Thus, cases and controls for preliminary pharmacogenetics studies may include the exposed (cases) and unexposed (controls), and among those exposed to the drug those with EP (true-cases) compared to those without EP (true-controls).

Once the sample size and phenotype are clearly determined, samples for a pharmacogenetic study can be collected in a clinical setting (here an ambulatory surgical center). Samples for this study were collected into EDTA tubes from existing intravenous (IV) line prior to removal of the line. In cases where aspiration of the IV access failed to return blood, direct venous puncture was performed by the PI. Leukocyte DNA is stable in EDTA at 4°C for 72

hours and even much longer when stored at –80degrees. The samples were collected over a 6 month period and stored at –80 degrees centigrade in a molecular pharmacology laboratory. DNA extraction and genotyping were out-sourced to university-based core laboratory facilities. Using the services of a core facility may not only improve the quality of the analysis, but equally reduce cost.

As expected, having a multi-disciplinary team was essential to the success of this pilot project. Our team included a nurse anesthetist PI, two nurse scientists (one with genetics expertise), and a biostatistician, with consultation from a molecular pharmacologist. In addition, advice was sought from physician anesthesiologists. This multi-disciplinary team ensured that all critical aspects of the study were possible. Prior to starting recruitment, the study was presented to surgeons, nurses, and anesthesia providers in the recruitment facilities. This buy-in was critical for recruitment of participants. Finally, potential participants were reassured that throughout the study, their clinical care superseded the research efforts.

Several limitations need to be acknowledged. First, internal validity of the study was diminished by the high rate of co-administration of midazolam (as a common anesthesia practice) among participants who received ketamine. As previously discussed, this could not be overcome in this current study design. Future studies should address this limitation in the design, as this pilot was not approved for a randomized controlled trial. Second, being a pilot study, the sample size was small and most of the patients were Caucasian, middle aged, and well-educated. These participant demographics limit the external validity or generalizability of the study findings. A larger sample is needed to increase the power to detect differences in genotype in future studies (see Table 5). It is possible that other, unstudied SNPs in the *CYP2B6* and *GRIN2B* genes may be associated with ketamine-induced EP. Unfortunately, no data were collected on ketamine metabolite concentrations, limiting our ability to draw direct conclusions about the influence of *CYP 2B6* genotype on ketamine metabolism.

Despite the limitations, this study had several strengths. Notably, participants were well characterized with stringent inclusive and exclusive criteria, thus reducing the number of variables that could affect ketamine metabolism (e.g. history of severe medical history i.e. ASA > II) and occurrence of EP (e.g. history of depression and schizophrenia). In addition, the CADSS tool was administered by one observer (PI), thereby reducing concerns of inter-observer variability. Finally, study occurred in a real clinical setting, which improves the generalizability of the findings.

Conclusions

This study provides effect size estimates to adequately power future studies investigating the relation between candidate gene variation and EP occurrence in the context of ketamine exposure. It also supports the feasibility of conducting a fully powered study in an ambulatory surgical setting investigating this important phenomenon. Our preliminary results supported some known evidence regarding nearly 9-fold increased risk of EP occurrence with ketamine exposure, and dose-dependent risk of EP occurrence and severity. Given these findings, close monitoring of patients undergoing or recovering from procedural

sedation with ketamine by nurses is strongly recommended. This is especially important for younger adults, receiving large doses of ketamine for relatively short procedures who are more likely to experience EP.

Conversely, some of our findings may offer evidence to potentially challenge current assumptions about patient satisfaction with care provided and EP occurrence. While patients experiencing EP may produce a negative experience for providers, and may be at risk of injury, ketamine induced EP did not affect patient reported satisfaction with care. This pilot study provided proof of feasibility as well as preliminary insights into the potential pharmacogenetic and non-genetic influences on ketamine induced EP. Further studies are necessary to improve the evidence behind this phenomenon.

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Figure 1.
Recruitment and sample flow

Table 1

Characteristics of study participants

Characteristics	No Ketamine Group		No EP Ketamine Group		EP Ketamine Group		P-values ^a	P-Values ^b
	Mean	SD	Mean	SD	Mean	SD		
	N = 28 (37.3%)		N = 28 (37.3%)		N = 19 (25.3%)			
Age (years)	51.89	13.57	51.04	13.02	47.84	16.04	0.522	0.456
Body Mass Index (kg/m ²)	29.99	6.61	27.23	5.43	28.28	4.98	0.095	0.503
Gender	N	%	N	%	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							0.455	0.651
White or Caucasian	22	78.6	26	92.9	16	84.2		
Asian	1	3.6	1	3.6	1	5.3		
Black or African American	2	7.1	1	3.6	1	5.3		
More than one Race	1	3.6	0	0	0	0		
Unknown/Not Reported	2	7.1	0	0	1	5.3		
Ethnicity (N, %)							0.599	1.000
Hispanic	3	10.7	2	7.1	2	10.5		
Non-Hispanic	24	85.7	26	92.9	17	89.5		
Education (N, %)							0.131	0.301
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	5	17.9	5	26.3		
Bachelor degree	3	10.7	8	28.6	3	15.8		
Master degree	2	7.1	2	7.1	5	26.3		
Professional/Doctorate	0	0	3	10.7	0	0		

P significant <0.1

^a: Ketamine cases versus Non-ketamine controls

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

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Table 2

Genotype and Allele Frequencies of Candidate SNPs

SNP	dbSNP ID	Allele (D/d) ^a	EP Positive (Cases)						EP Negative (Controls)						p-values		
			n ^b	HWE (p)	HWEX ²	Genotype	MAF	n ^b	HWE (p)	HWEX ²	Genotype	MAF					
			DD	Dd	dd				DD	Dd	dd						
CYP2B6	rs3745274	G/T	17	0.812	0.057	9	7	1	0.26	28	0.331	0.945	14	13	1	0.27	1.000
CYP2B6	rs2279343	A/G	16	0.364	0.825	9	5	2	0.28	28	0.604	0.269	13	13	2	0.30	0.578
GRIN2B	rs1019385	G/T	17	0.808	0.059	4	9	4	0.50	28	0.021	5.320	5	20	3	0.46	0.413
GRIN2B	rs1806191	G/A	17	0.466	0.529	5	7	5	0.50	28	0.700	0.148	7	15	6	0.48	0.725

* p<0.05

^aMajor/minor allele, major and minor alleles are denoted by D and d, respectively^bNumber of samples that are genotyped

Table 3

Using Logistic Regression to Predict EP Occurrence

Predictor	Wald <i>p</i> -value	OR	90% CI	Model χ^2	Model <i>p</i> 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	0.895 994				
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				
Genotype Predictor removed from Model							
Ketamine dose	0.002	1.135	1.063 1.211				
Age	0.093	0.952	0.907 0.999	18.251	0.001	0.435	76.6
Duration of Anesthesia	0.033	1.044	1.010 1.078				
Time to CADSS	0.020	0.949	0.915 0.985				

* - reference single nucleotide polymorphism genotype predictor

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CADSS- clinician administered dissociative state scale; Time to CADSS-time (minutes) from ketamine administration to assessment of EP; Age in years; Duration of anesthesia in minutes; ketamine dose in milligrams.

Table 4

Using Linear Regression to Prediction EP Severity

Predictor	B	β	P value	90% CI	F	Model P-value	R ²	Adjusted R ²
Ketamine dose (mg)	0.048	0.527	0.001	0.026	0.070			
Duration of Anesthesia (mins)	0.016	0.457	0.056	0.002	0.030	4.028	0.277	0.208
Age (years)	-0.025	-0.264	0.057	-0.046	0.004			
Time to CADSS (mins)	-0.019	-0.509	0.042	-0.340	-0.004			

P sig: < 0.1

EP severity measured as transformed (square root) total CADSS score

Table 5

Estimated Sample Size Requirement for Genetic Association Study

dbSNP ID	MAF	OR	Sample Size ^a	Adjusted OR ^b	Sample Size
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), assuming dominant genetic model

^b = Odds ratio of EP occurrence by genotype adjusted for ketamine dose, age, duration of surgery, time from ketamine administration to assessment of dissociation