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

**Methods:** This cross-sectional pharmacogenetic candidate gene study recruited 75 patients having minor 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). 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). 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. However, the small sample size may have limited about ability to determine significant genotype differences. This pilot study supports our approach, and we estimate that 380 and 570 cases will be needed to adequately power future genetic association studies. Despite concerns by providers, EP occurrence and severity does not appear to affect satisfaction with care provided.

**Keywords:** ketamine-induced emergence phenomena, pharmacogenetics, anesthesia, cytochrome P450, N-methyl-D-aspartate receptor antagonist.
A pilot study of the pharmacogenetics of Ketamine-induced emergence phenomena

Ketamine-induced emergence phenomena (EP) occurs 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 cingulated 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). 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 & Villevieille, 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 exert most of its desired and adverse effects by antagonizing the NMDAR.

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 (GRIN 2) 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) and many SNPs in that gene 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. Patients were excluded if they had a diagnosis of depression,

schizophrenia, or bipolar disorder. 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 $\geq 0.5\text{mg/kg}$ 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 -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 $\geq 4$ 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 $\geq 4$ 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). This deviation from HWE may be related to the small cell count given fact that this SNP has a MAF of 0.50 in the Caucasian population. 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
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

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). All models including the SNPs (rs3745274, rs2279343, rs1019385 & rs1806191) could reliably distinguish between EP cases and controls. Nagelkerke’s R² 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 R² of 21 percent. Age, dose of ketamine, duration of anesthesia and time since ketamine administration made significant contributions to the model (Table 4)
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.

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) might have limited our ability to demonstrate a significant association between ketamine induced EP occurrence and CYP2B6 (rs3745274 and rs2279343) and GRIN2B (rs1019385 and rs1806191) SNPs. These findings are consistent with previous studies that also did not find statistically significant 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, our findings extend theirs by demonstrating that the lack of association may not be
related to the fact that major mental disorders are known risk factors of EP—as we excluded patients with documented depression, bipolar disorder, and schizophrenia. In addition to low power, one potential reason for lack of association may be 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 makers, diabetic control (glycosylated hemoglobin), and obesity.

Interestingly, non-genetic variables (younger age, ketamine dose, duration of anesthesia and time from ketamine administration to assessment of dissociation) significantly predicted the occurrence and severity of ketamine induced EP. It is traditionally believed that EP is more common in females, in patients overs 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 findings emerged from this model: gender was not a significant predictor of EP; among adults, EP occurrence decreased with age; there was no significant interaction between ketamine dose and duration of anesthesia; and no interaction between duration of anesthesia and time from ketamine administration to assessment for dissociation. These findings suggest that all of the variables independently contributed to the occurrence of EP. 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. Thus, anesthesia providers should carefully titrate ketamine dose because administration of just 1ml (10mg) of ketamine increased the odds of developing EP three and half (3.52) times.

Another surprising finding from this pilot study is the fact that 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, Serinen, & 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, 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).

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. Samples for this study were stored in a molecular pharmacology laboratory and 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. 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 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 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 satisfaction with care. This pilot study provided proof of feasibility as well as preliminary insights into the potential pharmacogenetic influences on ketamine induced EP. Further studies are necessary to improve the evidence behind this phenomenon.
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