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GENETIC VARIATIONS OF CYP2B6 ENZYME AND THE RESPONSE TO MEPERIDINE IN ORAL SEDATION

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GENETIC VARIATIONS OF CYP2B6 ENZYME AND THE RESPONSE TO MEPERIDINE IN ORAL SEDATION

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Dentistry at Virginia Commonwealth University.

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Abstract

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By Sally Sang Guot Hua, B.A., D.M.D.

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Purpose: The purpose of this study was to determine the relationship of CYP2B6 genotype to the clinical response to meperidine in pediatric dental patients.

Methods: Twenty-five patients, ASA I/ II, 45–92 months old, received an oral sedative regimen containing meperidine for dental treatment. The North Carolina Behavior Rating Scale (NCBRS) and Overall Effectiveness of Sedation Scale (OESS) were used to assess their behavior and sedation outcome. Saliva DNA samples were genotyped by PCR-RFLP.

Results: We found the following genotype distributions: homozygous wild-type 1*1 (n = 8, 32%), heterozygous 1*6 (n = 13, 52%), and homozygous variant 6*6 (n = 4, 16%). The genotypes were predictive of a significant decrease in the overall effectiveness of sedation.

Conclusion: Variation in CYP2B6 appears to be predictive of less successful sedations; wild-type individuals experienced more successful sedations than the homozygous variant 6*6. Future research regarding the enzyme kinetics of meperidine is needed to determine the exact enzymatic function of CYP2B6 and its variants.

INTRODUCTION

According to the American Academy of Pediatric Dentistry (AAPD) moderate sedation (formerly known as conscious sedation or sedation/analgesia) is defined as "drug-induced depression of consciousness during which patients respond purposefully to verbal commands…either alone or accompanied by light tactile stimulation" [1]. In 1996, a survey by Wilson *et al* of 1758 AAPD members found that 40% of members use sedation 1 to 5 times per week and 20% use sedation more than 5 times per week.[2] It is estimated that more than 1 million children have been sedated by pediatric dentists since 1985.[2]

Meperidine (Demerol®) is commonly used for moderate sedation in pediatric dentistry. Meperidine's popularity in pediatric sedation is due to its fast on-set of approximately 15 minutes following oral administration. Peak sedation is achieved in approximately 2 hours and subsides over several hours. [3, 4] Meperidine is an opioid analgesic that was originally developed as an anticholinergic drug.[5, 6] It acts on the mu (μ) receptors found in the central nervous system (CNS) and on the neural elements in the bowel.[3, 7] Its opioid analgesic properties include inducing sedation, reducing reaction to painful stimuli and reducing motor activity.[3] Meperidine's side effects include hypotension, histamine release, nausea and vomiting, and decreased sensitivity to CO_2 leading to respiratory depression.[4, 7] Meperidine is primarily metabolized in the human liver by N-demethylation to form the active metabolite normeperidine (6-N-

desmethylmeperidine), which is a potent stimulant of the CNS with no analgesic properties.[7, 8] The accumulation of normeperidine can cause neurotoxicity and produce symptoms such as delirium, nervousness, tremor, muscle twitches and seizures.[7, 8]

Adverse drug reactions (ADRs) are a problem. Annually in the United States just over 2 million ADRs are estimated to occur, with approximately 100,000 resulting in death.[9] Meperidine may contribute to this problem is some patients. A review of pediatric dental adverse events and their contributing factors from 1969 through March 20, 1996 by Cote *et al.* found 95 reported incidents: 51 resulted in death, 9 in permanent neurologic injury, 21 in prolonged hospital stay without injury and 14 experienced no harm.[10] Twenty-nine of the 60 incidents resulting in death and permanent neurologic injury were related to various specialties in dentistry. [10]

In 2001, Leelataweedwud *et al.* examined 195 cases of conscious sedation in pediatric dentistry with the classic triple cocktail of chloral hydrate, meperidine and hydroxyzine.[11] The study found a success rate of 72%, while 23% were unsuccessful and 5% were aborted.[11] There were 3% with adverse events reported which included vomiting, desaturation, prolonged sedation and apneic episodes.[11] The incidence of meperidine ADRs is consistent with genetic variation being a partial causative factor.

Reducing ADRs is especially important when administering drugs to children in an outpatient setting. Outpatient procedures requiring children to receive sedation include gastrointestinal procedures, MRI scans, dental rehabilitation, and procedures completed in the emergency department. The most commonly used opioid analgesics for moderate sedation and analgesia are fentanyl and meperidine.[12] Common adverse effects of these

drugs, when used as single agents can include over-sedation, respiratory depression, mental clouding, delirium, seizures, hypotension, flushing, sweating and pruritis. While not lethal, these effects are common with and without significant co-morbidities. Practitioners today are unable to predict, without error, who will and who will not have an adverse drug reaction. Using pharmacogenomics to select medications could potentially increase therapeutic responsiveness from the 50% it is today to almost 75%, while dramatically reducing the number of ADRs occurring each year.[13] Pharmacogenetics could revolutionize pediatric sedation, and lead to increased patient satisfaction and safety.

The cytochrome P450 monooxygenase enzyme group is a multigene family of hepatic enzymes that are responsible for the oxidative metabolism of most medicines. Genetic variation in the metabolic activity of these enzymes can have a negative effect on drug efficacy and safety. Genetic polymorphisms in these and other enzymes can be used to guide drug treatment. Figure 1 shows the following isoenzymes which are responsible for the *in vitro* metabolism of the meperidine: CYP2B6, CYP3A4, and CYP2C19, with CYP2B6 being the major enzyme that metabolizes meperidine.[14] In addition its action in the liver, CYP2B6 has also been identified in the human brain.[15, 16]

The CYP2B6 gene is located on chromosome 19, between 19q12 and 19q13.2 and is composed of 9 exons.[17, 18, 19] Haplotype analysis demonstrates the presence of multiple alleles including the most common form or wild-type CYP2B6*1, and the most common variant, CYP2B6*6.[20] The activity of CYP2B6 varies between individuals and this variation has been shown to be genetic.[14] The diagnostic variant in the haplotype CYP2B6*6, is a single nucleotide polymorphism of G to T in exon 4 that results in a

substitution of Gln to His at amino acid 172 (516G>T, Gln172His). [21] This change is associated with a significant loss of function as measured by enzymatic activity.[21] This variation is clinically relevant. For example, the CYP2B6*6 variant has been reported to affect the pharmacokinetics of efavirenz (EFV), a first line medication for treatment of human immunodeficiency virus (HIV) patients.[20, 21] Patients who are homozygous CYP2B6*6 experience more adverse neurological events such as fatigue and mood disorders when they are put on long term EFV therapy compared to those who are homozygous wild type.[21]

In pediatric dentistry, we often encounter children who are unable to tolerate dental procedures comfortably despite traditional behavior management techniques and adequate local anesthesia. These children require sedation in order to receive care.[1] This group of patients, because of their age, is considered more susceptible to the adverse effects of sedatives and narcotics on the respiratory drive, loss of protective airway reflexes and airway obstruction.[1] Currently, oral sedative agent selection is based on the patient's behavior, weight, medical history, physical exam and anticipated duration of the dental procedures. Structured sedation protocols have shown to reduce morbidities and enhanced sedation safety for pediatric patients.[1] However there remains an element of unpredictability of response to sedation. One source of variability is thought to be genetics.

It is unknown at this time what affects the CYP2B6*6 allele may have on the pharmokinetics of meperidine. It may be associated with either an increase or decrease in enzymatic activity, which may have varying clinical effects such as slower drug clearance, resulting in prolonged sedation, or at the other end of the range excitability. The specific

aim of this study is to determine the relationship between the CYP2B6 genotype at this one loci and clinical response to meperidine in pediatric dental patients.

METHOD AND MATERIALS

Sample and data collection

Twenty-five patients previously identified as requiring oral sedation for dental treatment were recruited to participate in our research from the VCU School of Dentistry Pediatric Dental Clinic. Patient ages ranged from 45 to 92 months at the time of treatment. All patients were ASA I or II. The patients received an oral sedative regimen containing meperidine combined with one or more of the following medicines: chloral hydrate, hydroxyzine (Vistaril®) and midazolam (Versed®).

Informed consent for dental treatment under oral sedation, physical restraint and participation in the study were obtained from the parent/guardian. An assent form was obtained from patients who were 7 years or older for the saliva collection for CYP2B6 genetic testing. Saliva has been shown to be a viable and noninvasive method for obtaining DNA for genetic analysis.[22]

Prior to the administration of oral sedation medications baseline vital signs were obtained. After administration of the medications by the treating dental resident, the patients and their parents/guardian remained in the pre-op room for at least 30 minutes before the start of the dental procedure. Once in the treatment room, a blood pressure cuff and precordial stethoscope were applied and the patient was placed on a papoose board. Treatment began once all of the monitoring equipment was in place and the patient was

comfortable. The patient's heart rate, blood pressure, and oxyhemoglobin saturation rate (SaO_2) were recorded at five minute intervals. Respiratory status and breath sounds were monitored throughout the procedure via the precordial stethoscope by the treating pediatric dental resident.

The behavior of the child during the treatment was recorded using the North Carolina Behavior Rating Scale (NCBRS) and the overall effectiveness of the sedation was rated using the Overall Effectiveness of Sedation Scale (OESS).[23, 24] Vital signs, physiological parameters and behavior scores were charted by a monitoring provider. Once the AAPD discharge criteria[1] were met the parents/guardian were escorted into the treatment room to meet the patient. Post-operative instructions were given in verbal and written formats to the patients and their parents/guardian.

Adverse events were defined as follows: desaturation was when the pulse oximeter, SaO_2, reading was below 95%; apnea was when there is no breath sounds via precordial stethoscope and no visible sign of chest rise for greater than 25 seconds; excessive sedation was when the patient required more than 30 minutes to recover; seizure, nausea and vomiting.

Data collection was standardized prior to the start of this research. All nine residents and full-time faculty at the VCU Pediatric Dental Department were trained and calibrated by assessing 10 videotaped sedations that were not part of this study. The calibration videos were of patients of record at VCU Pediatric Dental Department who needed sedation for dental treatment. Informed consents for videotaping, physical restraint with a papoose board and standard treatment during oral sedation were obtained from the

parent/guardian. The calibration training entailed watching the videos of 10 taped sedations and assessing each patient's behavior based on the North Carolina Behavior Rating Scale (NCBRS) during critical events at every 5 minute intervals (see Appendix 1). The Overall Effectiveness of Sedation Scale (OESS) was used to rate the overall success of the oral sedation appointment ranging from "successful to unsuccessful" depending on how the patient's behavior affected the treatment outcome (see Appendix 1). The calibration study indicated significant agreement (Kappa = 0.60, $p < .0001$).[25]

Genetic analysis

For each patient, 2 ml of saliva was collected using Oragen DNA (OG-300) self DNA collection kit before and after the treatment. The patient's saliva was collected and the genetic analysis of CYP2B6 was done at a later date.

The DNA was extracted manually from 2ml of un-induced saliva by using QIAamp DNA Blood Mini Kit (Valencia, CA, USA), following the manufacturer's instructions. PCR amplified the exonic the *6 variable region of CYP2B6.[21]

The genotyping analysis was done with restriction fragment length polymorphism (RFLP). To generate the CYP2B6 526bp product, the following primers were used: 2B6*6F 5' - GGT CTG CCC ATC TAT AAA C - 3' and 2B6*6R 5' - CTG ATT CTT CAC ATG TCT GCG - 3'. The PCR product was digested with Fermentas BseNI restriction endonuclease enzyme. The digestion of the CYP2B6*6 variant allele 516TT amplicons yielded two fragments of 23 and 503 bp and that of the CYP2B6*1 wildtype allele 516GG amplicons resulted in 3 fragments of 23, 236 and 267 bp. The digestion products were

separated on a 2% aragose gel using electrophoresis, and banding patterns were visualized under UV light then photodocumented.

Statistical Analyses

To compare the observed genotype frequencies with those expected under Hardy-Weinberg equilibrium, a chi-square test with one degree of freedom was used. The primary aim was to test the association between CYP2B6 genotypes (homozygous for the normal allele = 1*1, heterozygous = 1*6, and homozygous for the variant allele = 6*6) and clinical response (behavior and sedation effectiveness), using data from the North Carolina Behavior Rating Scale and the Overall Effectiveness of Sedation Scale.

The groups were compared using a chi-square analysis for nominal outcomes and analysis of variance (ANOVA) for continuous outcomes. Multivariable analyses were accomplished using a repeated-measures mixed-model ANOVA (SAS software. JMP8.0 or SAS9.2, Cary NC). The study was approved by the Virginia Commonwealth University Institutional Review Board Committee on Investigations Involving Human Subjects. All clinical data were collected in the VCU Pediatric Dental Clinic and the DNA isolation was performed at the School of Pharmacy in the laboratory of Dr. Bukaveckas.

RESULTS

Preliminary analyses

The demographic characteristics of the patients (n = 25) are shown in Table 1. The patients were primarily African Americans (n = 19), with 5 Caucasians and 1 was marked of other race. There were 16 females and 9 males. The patient's ages ranged from 45 months to 92 months with an average age of 63.5 months at time of treatment. The majority of subjects (68%) were ASA I status, while the rest were ASA II. The mean time of treatment duration was 25.1 minutes with a range of 5 minutes to 63 minutes. The patients were categorized into three genotypes and identified as: 1*1 for homogenous wild-type allele CYP2B6 (n = 8, 32%), 6*6 for homogenous variant allele (n = 4, 16%), and 1*6 for heterozygous allele (n = 13, 52%). These proportions were not different than the expected values (25%, 50%, 25%, chi-square = 1.32, df = 2, $p > 0.5$) under the Hardy-Weinberg equilibrium. Comparing the demographic characteristics in Table 1, there were no significant differences between the genotypes (*p*s > 0.09).

The medications used in the patients are described in Table 2. The triple-cocktail combination of meperidine, midazolam, hydroxyzine was used in 68% of the cases. The second most common drug regimen was meperidine, midazolam, and chloral hydrate, used in 20% of the cases. The meperidine and midazolam combination and meperidine and

hydroxyzine combination were each used once. In one case, Propofol® was used after converting to intravenous sedation (IVS) due to failed oral sedation.

In the study cohort (n=25), 48% received restorations (n = 12), 12% extractions (n = 3), 28% both restorations and extractions (n = 7), and in 12% of the cases the planned procedures were not performed and the process was aborted (n = 3). There were no instances of apnea or nausea, one instance of vomiting, two instances of desaturation and three instances of excess sedation meaning the patients too longer than 30 minutes post-op for recovery.

Primary analyses

The primary goal of the study was to compare the overall effectiveness of oral sedation between three genotype groups: CYP2B6*1*1, CYP2B6*1*6 and CYP2B6*6*6. Table 1 shows the number of individuals in each genotype and sedation effectiveness combination. The genotype groups showed a significant difference in the overall effectiveness (Wilcoxon rank-sum chi-square = 10.3, df = 2, p = 0.0058). As may be seen in the table, the CYP2B6*1*1 genotype had the most effective success scores (median effectiveness = 2) while the homozygous variant, CYP2B6*6 genotype had the worst (median = 4).

A stepwise regression analysis of the demographic characteristics and drug regimens was performed to determine if the difference between genotypes could also be explained by a confounding factor. Only Vistaril (p = 0.17) and Propofol (p = 0.17)

emerged as potential confounders (using an alpha cut-off of 0.2). Including these in the model did not change the conclusion that effectiveness differed by genotype.

Secondary analyses

The secondary analysis focused on outcomes that were assessed on repeated occasions during the course of each child's procedure. These outcomes were: NCBRS, HR, Dia-BP, Sys-BP, and SaO_2. Each of these outcomes were analyzed separately with a repeated-measures ANOVA with the following factors in the model: Event type (Baseline, preOp, IntraOp, and PostOp), genotype (the three values), and an event*genotype interaction.

The NCBRS was recorded on 168 occasions (between 0 and 14 times per patient) and had a mean = 1.95, SD = 1.10. NCBRS was not assessed during the post-operative period. The results of the repeated-measures ANOVA indicated that NCBRS did vary across event types ($p < .0001$), that the genotypes did differ ($p = 0.0064$) and that the event differences did not vary with genotype ($p > .5$, see Table 4). The estimated mean NCBRS for each genotype and event is also shown in Table 4 and Figure 2. At baseline, the three genotypes are not significantly different (uncorrected p-value = 0.22) but at pre-operative phase (PreOp) they have become different ($p = 0.0410$). At the intraoperative period (IntraOp), the genotypes are different ($p = 0.0007$). Within the genotype 1*1, there was no difference across the events ($p = 0.14$) but within the 1*6 genotype there was a significant trend ($p = 0.0020$) and within the 6*6 group as well ($p = 0.0035$).

The 1*1 individuals behaved with an NCBRS of 1.14 to 1.64, meaning they were relatively quiet and had some inconsequential movements throughout the procedures. While at the other end, those with genotype 6*6 had an average NCBRS of 2.25 baseline and 3.71 intraoperatively. Those who possess 1*6 genotype, their NCBRS was 1.46 at baseline and 2.27 during intra-op, which are between the values of those that have 1*1 and 6*6 genotypes.

Another way to perform this comparison is to consider the NCBRS as an ordered multinomial response. The number and percentage are shown in Table 5. The traces for each patient in each genotype group are shown in Figure 2. As is seen, in the genotype 1*1 group, all but one subject are NCBRS=1 at baseline and PreOp and only a few of the subjects increase averages between 2-3 by IntraOp. The 1*6 group have traces that also begin in the 1-2 range and then increase to the 2-3 range, or in some cases as high as NCBRS=3 or 4. There are only n = 4 subjects in the 6*6 genotype and many seem to begin at higher levels and all end in averages in ranges near 3-4.

The ordered multinomial outcomes may be modeled using a cumulative logit and the GEE method for accounting for repeated measures (SAS GENMOD procedure). As is seen in Table 6, there remain differences between the Events ($p < 0.03$) but the genotype difference is less clear ($p > 0.07$).

The heart rate, (HR) was recorded on 300 occasions (between 2 and 19 times per patient) and had a mean = 99.7, SD = 20.1. The results of the repeated-measures ANOVA indicated that HR did vary across event types ($p < .0001$), that the genotypes did not differ ($p > 0.5$) and that the event differences did not vary with genotype ($p > 0.5$, see Table 3).

The estimated mean heart rate for each event is also shown in Table 7 and Figure 6. Tukey's HSD indicated that the PreOp mean was not significantly different than any of the others and that each of the others was significantly different from one another.

The systolic blood pressure (Sys-BP) was recorded on 285 occasions (between 2 and 18 times per patient) and had a mean = 121.5, SD = 21.2. The results of the repeated-measures ANOVA indicated that Sys-BP did vary across event types ($p = .004$), that the genotypes did not differ ($p > 0.8$) and that the event differences did not vary with genotype ($p > 0.7$, see Table 8). The estimated mean systolic BP for each event is also shown in Table 8 and Figure 7. Tukey's HSD indicated that only the PreOp and IntraOp values were significantly different from one another.

The diastolic blood pressure (Dia-BP) was recorded on 285 occasions (between 2 and 18 times per patient) and had a mean = 67.5, SD = 13.8. The results of the repeated-measures ANOVA indicated that Dia-BP did vary across event types ($p = .002$), that the genotypes did not differ ($p > 0.8$) and that the event differences did not vary with genotype ($p = 0.152$, see Table 5). The estimated mean dia-BP for each event is also shown in Table 9 and Figure 7. Tukey's HSD indicated that the IntraOp and PostOp values were not different from one another, but that they were significantly higher than PreOp. Baseline values were not different than any other event.

The oxygen saturation, SaO_2, was recorded on 296 occasions (between 2 and 19 times per patient) and had a mean = 98.6, SD = 1.4. Since SaO_2 was so strongly skewed, with 90% of the values above 98, this outcome was analyzed on the log-scale and then the summary results back transformed to the original scale. The results of the repeated-

measures ANOVA indicated that SaO_2 did not vary across event types ($p > .2$), that the genotypes did not differ ($p > 0.6$) and that the event differences did not vary with genotype ($p > .7$, see Table 10). The estimated mean SaO_2 for each event is also shown in Table 10 and Figure 8. There were two cases where patients experienced desaturation (<95% SaO_2).

DISCUSSION

Genetic finding:

In vitro studies of CYP2B6 have shown that all variant alleles encode functionally active proteins.[26, 27] The mean protein expression level of those who were heterozygous, 1*6, compared to that of the wild type, 1*1, did not show a significant reduction (Lang et al 2000). However, there was a reduction of approximately 50% in protein expression for those who were homozygous 6*6.[26] This was as expected from a clinical efavirenz (EFV) study where they found that homozygous for the *6 variant allele had more than three-fold higher plasma drug concentration than those who were wild types.[21] In a study by Rodriguez-Novoa *et al.* 40% homozygous 6*6 and 19% of hetrozygous had EFV concentration >4μg/mL, which is the toxic level. Nearly 20% of homozygous 1*1 and 2% of homozygous 6*6 showed subtherapeutic level of EVF of <1μg/mL.[28] The clinical relevance to their research was the individuals who carried the wild type allele had subtherapeutic concentration of EFV and were at risk for treatment failure; in contrast, those who were homozygous 6*6 experienced neurological adverse effects more frequently. As expected, a reduction in enzymatic function was more likely to lead to an accumulation of EFV plasma concentration within the toxic range.

The homozygous variant CYP2B6*6, homozygous wild-type CYP2B6*1, and heterozygous CYP2B6 genotypes were present in 16%, 32% and 52% of our population, respectively. There were no statistical significant differences found between the

demographic characteristics and the genotypes (ps > 0.09). Interestingly, our study results showed the opposite of what was expected based on *in vitro* studies of CYP2B6 function. There was a statistical significance in overall effectiveness of sedation outcome, (chi-square = 10.3, df = 2, p = 0.0058) between the genotypes and their overall sedation success. Table 3 showed that the homozygous wild-type, 1*1, had an average of overall effectiveness score of 2, which translated to a moderately successful sedation with moderate amount of crying and movement. In patients who were homozygotes for 6*6, they had a mean score of 4 which was interpreted as an unsuccessful sedation outcome, with continuous crying and movements throughout sedation, treatment performed with difficulty, and treatment progression was hindered.

One possible explanation to the phenotypes observed in our study was the possibility of one amino acid substitution of Gln172His mutation caused by natural single-nucleotide polymorphism enhancing the enzymatic activity of CYP2B6*6. Ariyoshi *et al in vitro* enzyme kinetic study demonstrated that wild-type CYP2B6 followed the classical hyperbolic Michaelis-Menten kinetics while the variant allele CYP2B6*6 showed the sigmoidal kinetics with a higher Vmax value compared to that of the wild-type enzyme.[29] Sigmoidal kinetics plot indicates cooperative binding of substrate to the active site which means that the binding of one substrate molecule affects the binding of subsequent substrate molecules. Allosteric activation by its substrate, also called homotropic cooperativity, is also seen in CYP3A4 mediated drugs metabolism.[29] This autoactivation phenomenon appears dependent on the substrate.[29]

CYP2B6*6 catalytic activity may be enhanced with meperidine. This would explain the phenotypes observed in our study population. The patients who were homozygous 6*6 may have metabolized meperidine at a faster rate, leading to accumulation of normeperidine, which is associated with symptoms of neurotoxicity and CNS excitation. Furthermore, blood levels of normeperidine:meperidine AUC ratio is higher when it is delivered orally compared to the parenteral route.[30, 31, 32] While delirium, tremor, muscle twitches and seizures did not occur in the study, the NCBRS for patients with the variant allele were classified as "wild" meaning defiant with undesirable behaviors (crying, screaming, head movement, torso movement, hand movement or foot movement at critical events). Such phenotypes can be interpreted as symptoms of CNS stimulation by normeperidine.

It appears that CYP2B6 and its variants activity may not be generally predictable by genetic diagnosis and is dependent upon their substrate. Our research showed that future investigations will be needed to exactly determine the enzyme CYP2B6*6 properties toward meperidine. Future studies involve CYP2B6 variants and meperidine pharmacokinetics may help to explain whether there is an increase in normeperidine concentration in plasma and in peripheral blood mononuclear cells due to enhanced enzymatic activity caused by autoactivation.

Behavioral findings

This study design fostered a reliable behavior assessment since each rater (dental resident/faculty) was calibrated using the NCBRS and the OESS scales. The stepwise

analysis of the demographic characteristic and drug regimens was performed and shown that the drug regimen (Table 2) did not change the conclusion that the overall effectiveness differed by genotype. At baseline, the NCBRS did not differ between the three genotypes. However, at intraoperative phase, there was a difference between 3 groups as shown in Table 4 and Figure 9. Within the wild-type allele, there was no difference across the events ($p=0.14$). However, within the 1*6 and 6*6 variant alleles, there was a significant trend difference in the events, $p = 0.0020$ and $p = 0.0035$, respectively. The NCBRS for group 1*1 started at 1 at baseline and increased to 2-3 by intraoperative phase compared to group 1*6 and 6*6 which ended with a rating of 3-4 during intraoperative phase.

The overall effectiveness of sedation score may be high for some patients. If the patient was extremely vocal during the intraoperative length of the treatment, the treating dentist may have rated the sedation in a more negative manner despite the fact the child remained still and treatment proceeded without complications.

Physiologic findings

In the study population (n=25), 12% were aborted due to the patient's behaviors. Adverse events were reported as followed: 3 cases of excessive sedation (>30 minutes for recovery), 1 case of vomiting, and 2 cases of desaturation. There were no instances of apnea or nausea. In pediatric patients, nausea does not always procede vomit, which could occur instantaneously without warning.

In oral sedation, pediatric dental patients often cry and struggle during treatment therefore it is not uncommon to see "false alarms" meaning oxygen desaturation associated

with movements. These “false alarms” should not be overlooked. In oral sedation, desaturation, when the pulse oximeter reading is <95%, could happen due to many reasons including hypoxia, hypoventilation, excessive patient movements that cause mechanical interference, or pressure that the operator exerts on the mandible creating a mechanical airway obstruction. In our study, the desaturation was found in two cases which was promptly adjusted back to normal readings of >95% SaO_2 saturation after adjusting the position of the mandible and the pulse oximeter monitor.

Vital signs (heart rate, BP, and SaO_2) were not statistically significant between the different genotypes. The tendency for heart rate to increase with different event types, such as baseline to intraoperative phase, was seen. Such a finding can be explained as during intraoperative phase, which was when the patient was stimulated with local anesthetic injection, rubber dam placement and dental operative procedures, the heart rate could increase. Of critical importance was the average heart rate, 124.2 beats/minute, through out the sedation fell within the normal range for children age 3 – 5, which is 80-125 beats/minute. In addition, the average systolic pressure was 98.52 during intraoperative phase, which is also within the normal limits of systolic pressure for children age 3 – 5, which is 100 mmHg.

CONCLUSION

Many studies have focused on parameters maximizing sedation success while minimizing ADRs associated with oral sedation medicines. However, at this time, no studies have looked into the genetic component to oral sedation medicine, specifically meperidine, and the sedation outcomes. We found that after the administration of oral sedation regimens containing meperidine, individuals who carry the homozygous allele CYP2B6*6 had less successful sedation outcomes and less desirable behaviors compared those who were wild-type and heterozygous, who experienced better sedation outcomes. While meperidine, at the recommended dosage, is considered safe for oral sedation, the usefulness of CYP2B6 genetic analysis to personalize medicine may increase patient safety and satisfaction.

Genotyping patients for the variant allele CYP2B6*6 prior to receiving meperidine as an oral sedative for dental treatment in young children may prove to be important for identifying individuals with genetic predisposition for sedation failure, unnecessary anesthesia risks, economical, physical and emotional distress for both the child and the parent. Further research investigating CYP2B6 and its variants exact enzymatic function with respect to meperidine will contribute to the clinical significance of this enzyme.

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Table 1: Demographic Characteristics

Characteristic	n	%
Race		
AA	19	76
C	5	20
O	1	4
Ethnicity		
H	2	8
N	23	92
Gender		
F	16	64
M	9	36
ASA		
1	17	68
2	8	32
Wt (kg)		
Mean	23.0	
SD	6.5	
Min.	15	
Max	38	
Duration of tx (min)		
Mean	25.1	
SD	17.0	
Min.	5	
Max	63	
Age (years)		
Mean	4.8	
SD	1.1	
Min.	3	
Max	7	
Age (months)		
Mean	63.5	
SD	13.1	
Min.	45	
Max	92	

Table 2: Medications

Medications	n	%
Drug regime		
Dem Vis Ver	17	68
Dem Vis CH	5	20
Dem Ver	1	4
Dem Vis	1	4
Dem Vis Ver CH Pro	1	4
Local		
none	6	24
L+epi	11	44
L+epi Sept	2	8
Sept	6	24
N2O use		
N2O	21	84
none	4	16

Abbreviations: Dem = Demerol, Vis = Vistaril, Ver = Versed, CH = Chloral hydrate, Pro = Propofol, L = Lidocaine, Sept = Septocaine, N20 = Nitrous oxide.

Table 3: Comparing Overall Effectiveness

CYP2B6 genotype	Overall Effectiveness				
	1	2	3	4	Median
1*1	3	3	2		2
1*6		4	4	5	3
6*6			1	3	4

Table 4: Analysis of NCBRS

Source	df Num.	df Den.	F	p-value
Event	2	141.40	12.90	<.0001
Genotype	2	25.36	6.20	0.0064
Event*Genotype	4	141.40	0.77	0.5437

Event	LS Mean	SE	95% CI	
Genotype 1*1				
Baseline	1.14	0.38	0.39	1.90
PreOp	1.14	0.38	0.39	1.90
IntraOp	1.64	0.26	1.10	2.19
Genotype 1*6				
Baseline	1.46	0.31	0.84	2.07
PreOp	1.55	0.29	0.98	2.13
IntraOp	2.27	0.20	1.85	2.70
Genotype 6*6				
Baseline	2.25	0.50	1.25	3.25
PreOp	2.75	0.50	1.75	3.75
IntraOp	3.71	0.38	2.94	4.49

Table 5: Observed Counts and Percentages for each category of NCBRS outcome.

	NCBRS				
Event	1	2	3	4	Total
	Genotype 1*1 (n = 7)				
Baseline	6 (86%)	1 (14%)	0 (0%)	0 (0%)	7
PreOp	6 (86%)	1 (14%)	0 (0%)	0 (0%)	7
IntraOp	28 (61%)	10 (22%)	5 (11%)	3 (7%)	46
Total	40 (67%)	12 (20%)	5 (8%)	3 (5%)	60
	Genotype 1*6 (n = 13)				
Baseline	6 (60%)	3 (30%)	1 (10%)	0 (0%)	10
PreOp	8 (67%)	2 (17%)	1 (8%)	1 (8%)	12
IntraOp	25 (41%)	13 (21%)	16 (26%)	7 (11%)	61
Total	39 (47%)	18 (22%)	18 (22%)	8 (10%)	83
	Genotype 6*6 (n = 4)				
Baseline	1 (25%)	1 (25%)	2 (50%)	0 (0%)	4
PreOp	1 (25%)	0 (0%)	2 (50%)	1 (25%)	4
IntraOp	2 (12%)	2 (12%)	2 (12%)	11 (65%)	17
Total	4 (16%)	3 (12%)	6 (24%)	12 (48%)	25

Table 6: Multinomial Analysis of NCBRS

Source	df	Chi-sq.	p-value
Event	2	7.41	0.0246
Genotype	2	5.19	0.0748
Event*Genotype	4	3.16	0.5306

Predicted Percentages for each outcome:

	NCBRS				
Event	1	2	3	4	Total
	Genotype 1*1 (n = 7)				
Baseline	86	8	4	1	100
PreOp	86	8	4	1	100
IntraOp	61	20	13	6	100
	Genotype 1*6 (n = 13)				
Baseline	64	19	12	5	100
PreOp	66	18	11	5	100
IntraOp	40	25	23	13	100
	Genotype 6*6 (n = 4)				
Baseline	32	24	27	17	100
PreOp	18	19	32	31	100
IntraOp	6	9	25	60	100

Table 7: Analysis of Heart Rate

Source	df Num.	df Den.	F	p-value
Event	3	271.40	8.21	<.0001
Genotype	2	25.68	0.55	0.5863
Event*Genotype	6	271.10	0.88	0.5098

Event	LS Mean	SE	95% CI	
Baseline	88.30	4.36	79.62	96.98
PreOp	95.42	4.53	86.41	104.42
IntraOp	104.32	3.42	97.32	111.32
PostOp	98.55	3.44	91.51	105.59

Table 8: Analysis of Systolic Blood Pressure

Source	df Num.	df Den.	F	p-value
Event	3	255.00	4.52	0.0042
Genotype	2	23.62	0.12	0.8849
Event*Genotype	6	254.70	0.64	0.7007

Event	LS Mean	SE	95% CI	
Baseline	113.86	5.31	103.29	124.42
PreOp	112.78	4.89	103.01	122.56
IntraOp	124.02	3.96	115.90	132.14
PostOp	118.11	4.00	109.91	126.31

Table 9: Analysis of Diastolic Blood Pressure

Source	df Num.	df Den.	F	p-value
Event	3	258.80	5.02	0.0021
Genotype	2	27.71	0.14	0.8686
Event*Genotype	6	258.60	1.59	0.1515

Event	LS Mean	SE	95% CI	
Baseline	62.46	3.35	55.85	69.08
PreOp	61.18	3.01	55.22	67.13
IntraOp	68.83	2.15	64.46	73.20
PostOp	70.31	2.18	65.88	74.75

Table 10: Analysis of Oxygen Saturation

Source	df Num.	df Den.	F	p-value
Event	3	272.50	1.40	0.2421
Genotype	2	31.77	0.38	0.6847
Event*Genotype	6	272.70	0.62	0.7139

Event	LS Mean	95% CI	
Baseline	98.57	97.91	99.24
PreOp	98.23	97.54	98.92
IntraOp	98.52	98.09	98.94
PostOp	98.83	98.40	99.25

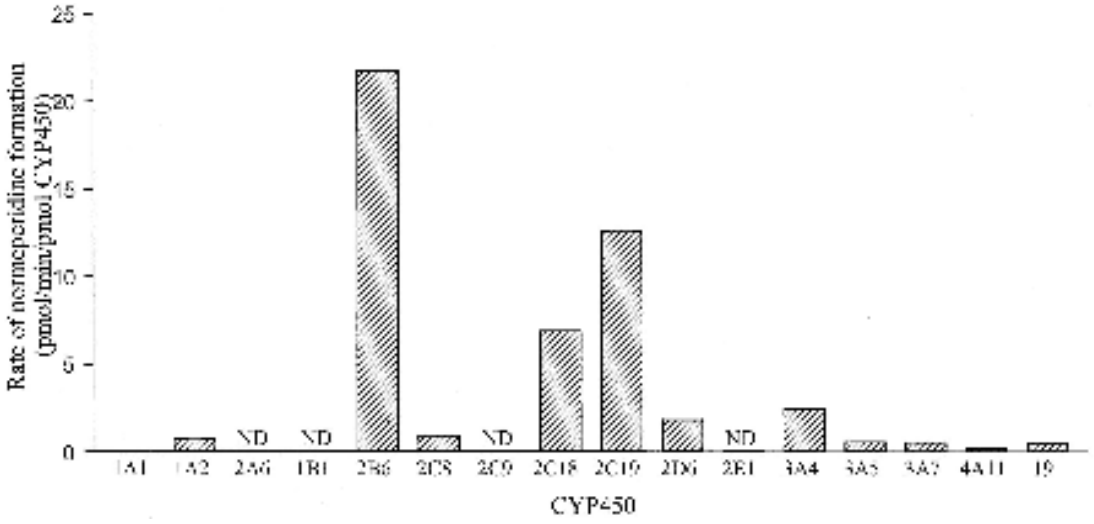

Figure 1. CYP450 isozymes responsible for meperidine metabolism. As illustrated in the figure, it has been demonstrated *in vitro* that Cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6) is the enzyme primarily responsible for metabolism of meperidine [14]

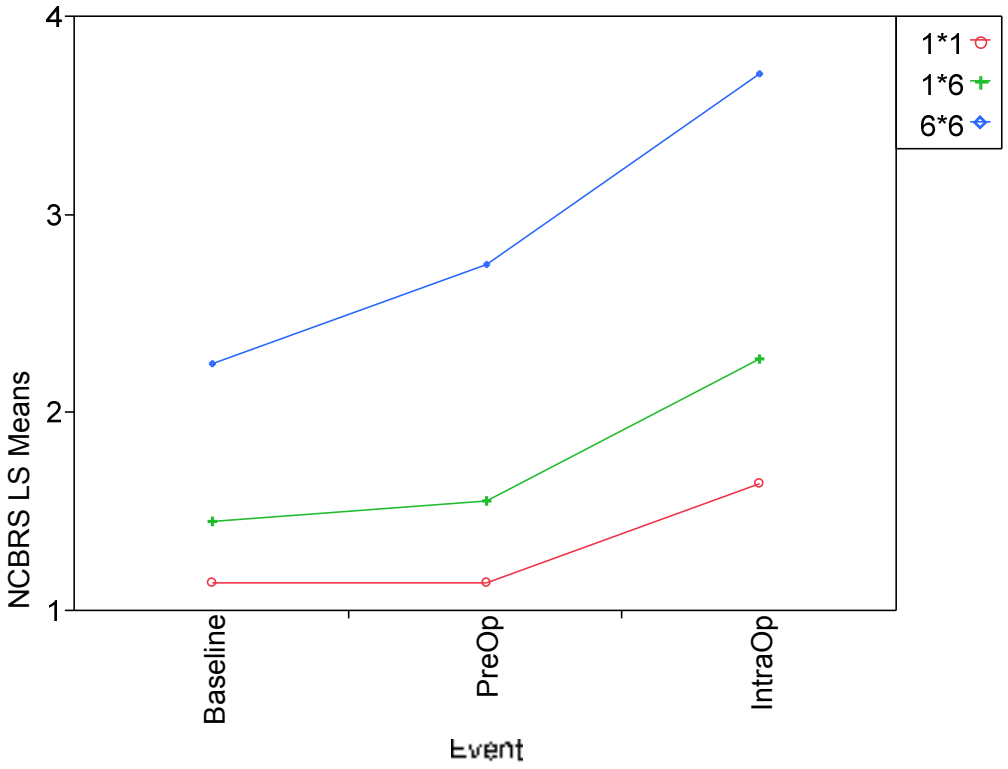

Figure 2: NCBRS

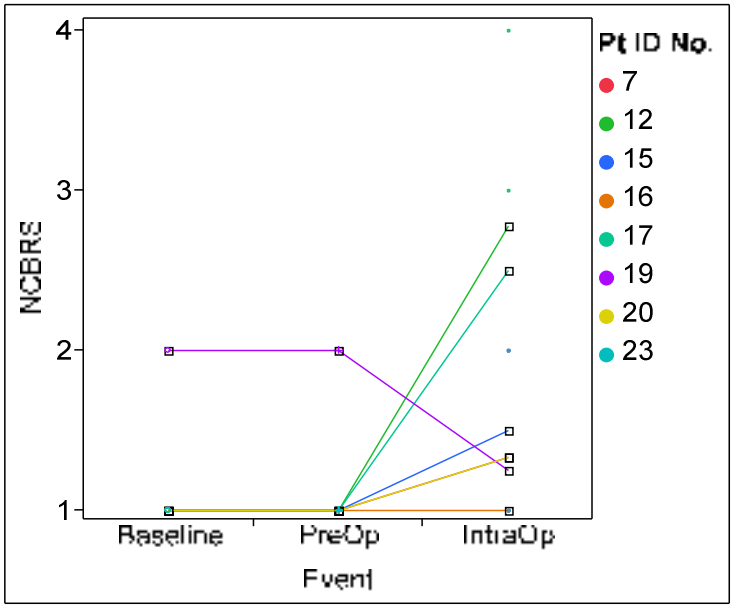

Figure 3: Traces of NCBRS for patients with genotype 1*1

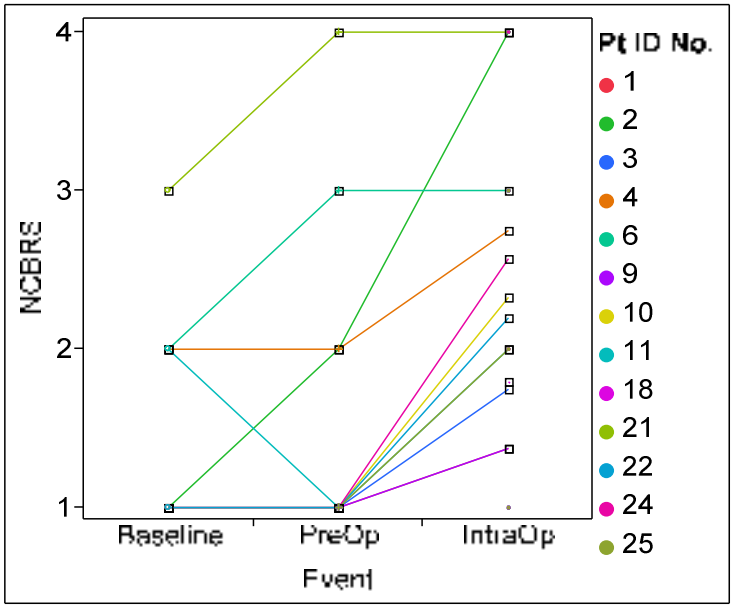

Figure 4: Traces of NCBRS for patients with genotype 1*6

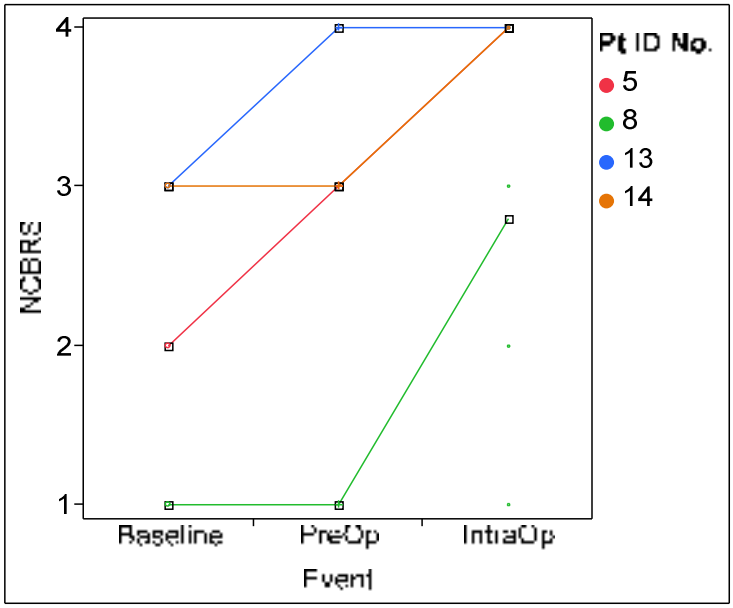

Figure 5: Traces of NCBRS for patients with genotype 6*6

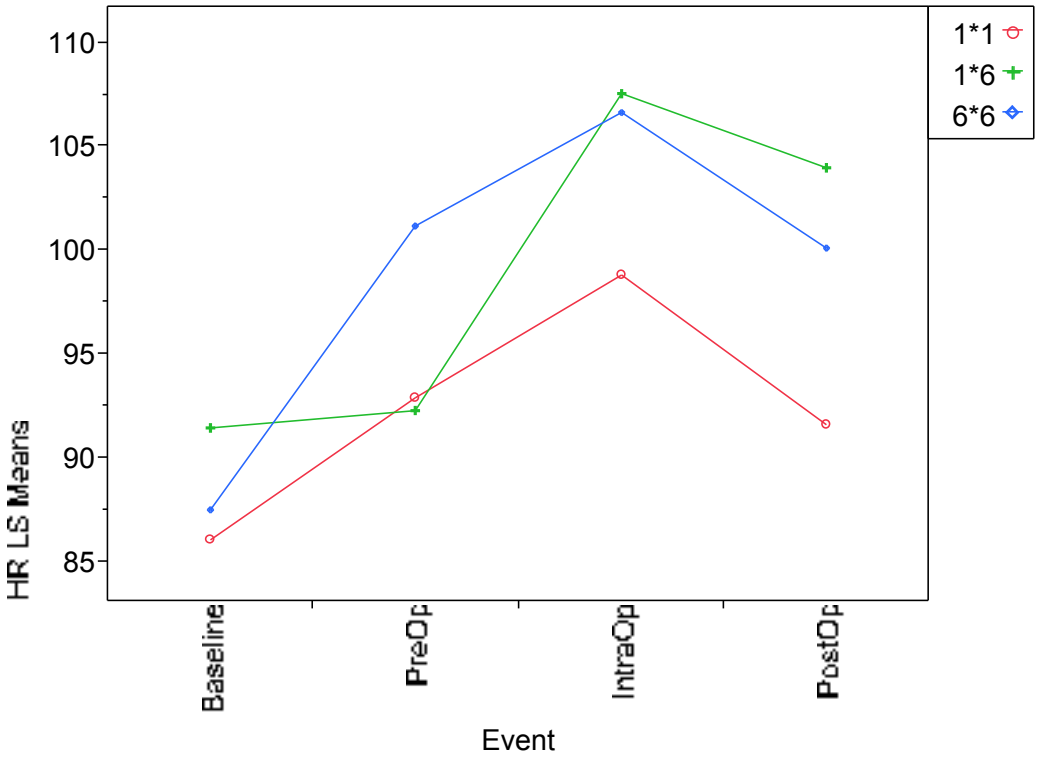

Figure 6: Analysis of Heart Rate

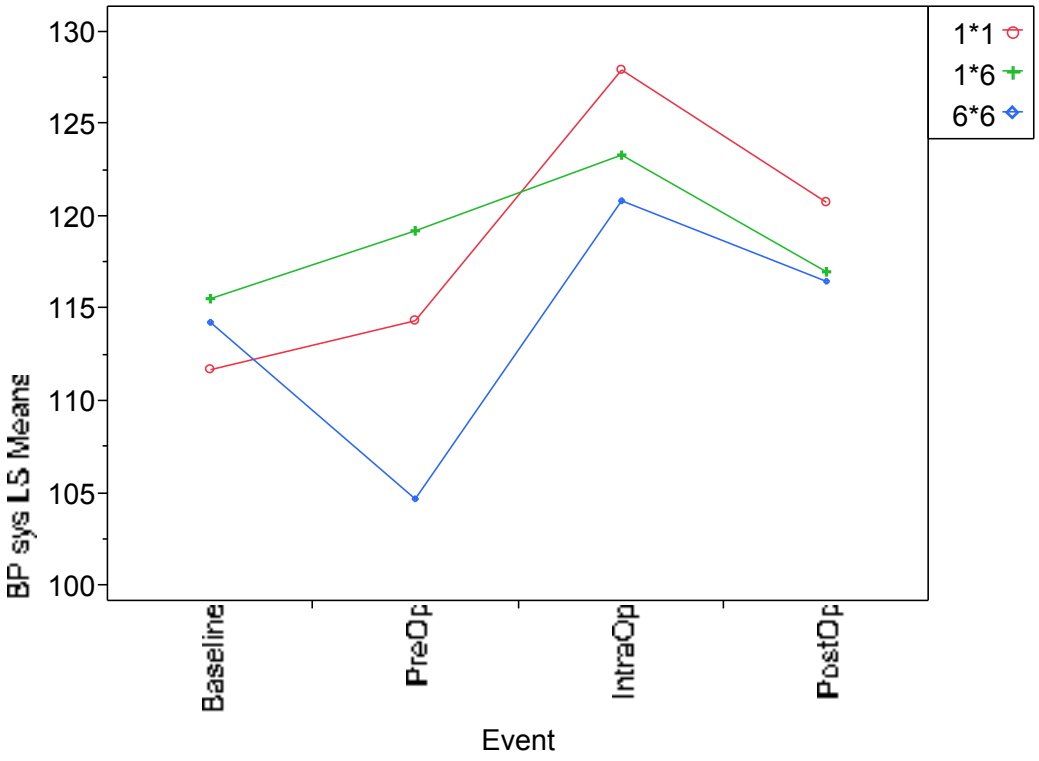

Figure 7: Systolic Blood Pressure

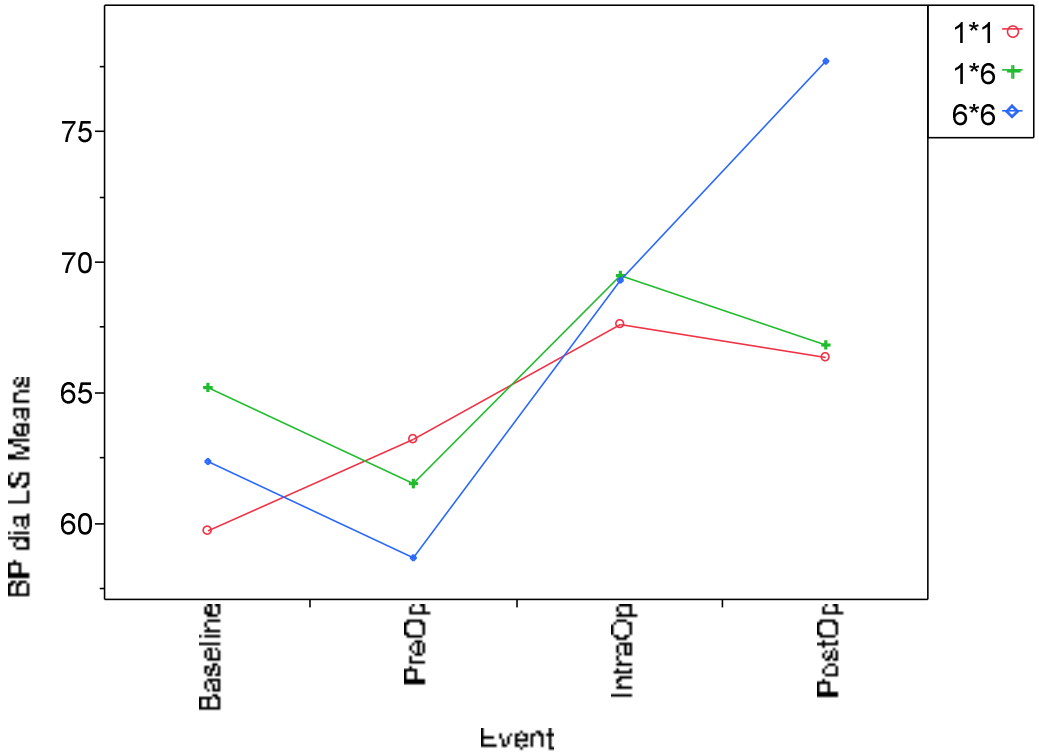

Figure 8: Diastolic Blood Pressure

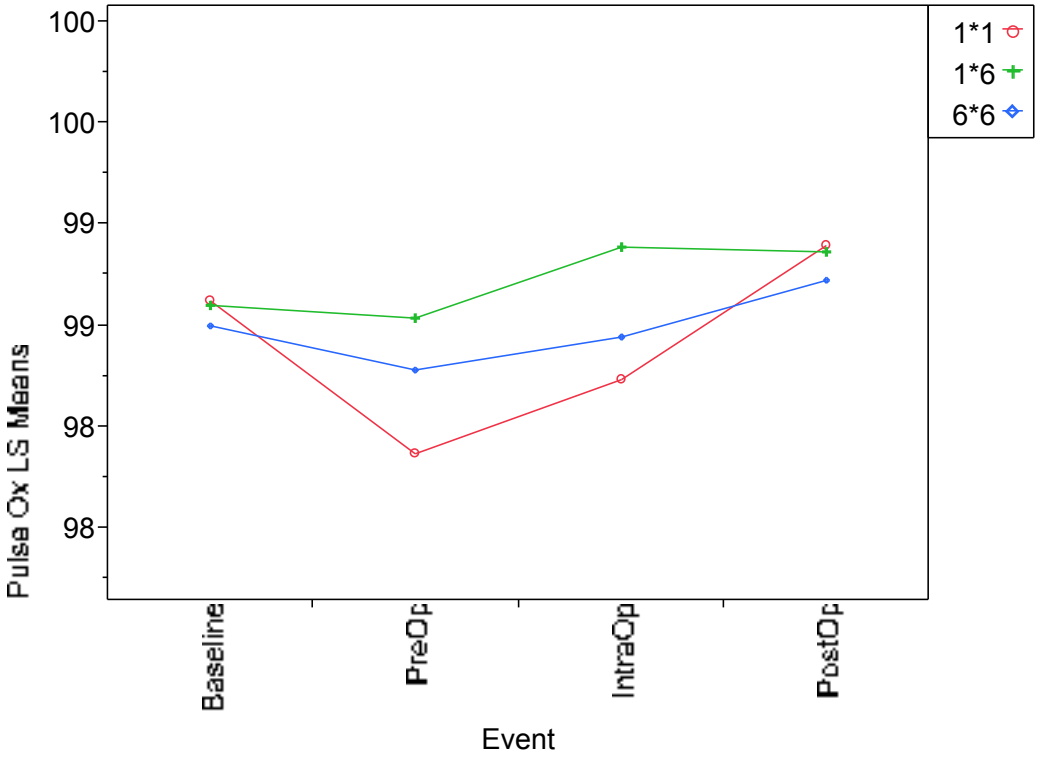

Figure 9: Pulse Oxygen

APPENDIX A

Behavior rating scales

The North Carolina Behavior Rating Scale and Overall Effectiveness of Sedation Scale were used to assess clinical response to meperidine and compare the relationship of CYP2B6 genotype and clinical response to meperidine. This appendix serves as a description of these scales.

North Carolina Behavior Rating Scale (behavior):

The North Carolina Behavior Rating Scale (NCBRS) allows the practitioner and assistant to assess behavior at critical events of the procedure. Behavior ranging from quiet and cooperative (1) to wild and defiant (4) is scored at critical events.**

1. Quiet: patient is quiet and/ or sleeping with only extraneous, inconsequential movements
2. Annoyed: patient is cooperative for treatment, but with one or two of the undesirable behavior*
3. Upset: patient is noticeably disturbed, with two to three undesirable behaviors* making treatment difficult but possible
4. Wild: patient is extremely defiant with presence of all undesirable behaviors* making treatment extremely difficult.

* An undesirable behavior includes crying, screaming, head movement, torso movement, hand or foot movements at critical events**

** Critical events: local anesthetic delivery (L), rubber dam placement (R), operative phase (O) such as bur penetrating tooth to rubber dam removal and extraction, IV conversion (C).

Overall Effectiveness of Sedation Scale.

1. Successful: Patient slept throughout procedure with only minimal crying/ movement at critical events*
2. Moderately successful: Successful sedation with moderate amount of crying and movement but behavior did not hinder the progress of sedation
3. Mildly successful: Treatment was accomplished as planned, but due to screaming/ combative movements throughout the sedation; the progression of portions of the treatment were hindered
4. Unsuccessful: Continuous crying/movement throughout sedation; treatment was performed with difficulty; the progression of all treatment was hindered

VITA

Sally Sang Guot Hua was born on January 13th, 1980 in Saigon, Vietnam. In 2000, she became a naturalized citizen of the United States of America. She graduated from Braintree High School, Braintree, MA in 1998. She attended Mount Holyoke College, South Hadley, MA and received her Bachelor of Arts, *cum laude*, in Biology in 2002. She also obtained a graduate certificate in Information Technology from the University of Queensland, Brisbane, Queensland, Australia in 2003. Dr. Hua, a Dean Scholar, received her Doctor of Dental Medicine from the University of Pennsylvania School of Dental Medicine in May of 2007.