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School of Dentistry
Virginia Commonwealth University

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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.

by

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June 2010

Abstract

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

by Heath Austin Whitfield D.D.S.

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

Virginia Commonwealth University, 2010

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

Methods: Forty-nine patients, ASA I/ II, 41–101 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 = 19, 39%), heterozygous 1*6 (n = 25, 51%), and homozygous variant 6*6 (n = 5, 10%). The genotypes showed a significant difference in the North Carolina Behavior Rating Scores and a trend towards significance of the Overall Effectiveness of Sedation Scale during meperidine oral sedations.

Conclusion: This research concludes that variations of the CYP2B6 enzyme can be used in the prediction of successful behaviors for oral sedations that include meperidine in the drug regimen. Future research regarding the enzyme kinetics of meperidine is needed to determine the exact enzymatic function of CYP2B6 and its variants.

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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 pharmacokinetics 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 behavioral responses to meperidine in pediatric dental

patients. A secondary aim was to examine physiological parameters during the oral sedation according to genotype.

METHOD AND MATERIALS

Sample and data collection

Forty-nine 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 41 to 101 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 was 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 *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 wild type allele 516GG amplicons resulted in 3 fragments of 23, 236 and 267 bp. The digestion products were separated on a 2% agrose gel using electrophoresis, and banding patterns were visualized under UV light then photo documented.

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. Clinical data was collected in the VCU Pediatric Dental Clinic and the DNA isolation was performed at the VCU School of Pharmacy in the laboratory of Dr. Bukaveckas and PGXL Laboratories (Louisville, KY, USA).

RESULTS

Preliminary analyses

The demographic characteristics of the patients (n = 49) are shown in Table 1. The patients were primarily African Americans (n = 32), with 12 Caucasians, 1 Asian and 4 were marked of other race. There were 28 females and 21 males. The patient's ages ranged from 41 months to 101 months with an average age of 63.1 months at time of treatment. The majority of subjects (80%) were ASA I status, while the rest were ASA II. The mean time of treatment duration was 24.8 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 = 19, 39%), 6*6 for homogenous variant allele (n = 5, 10%), and 1*6 for heterozygous allele (n = 25, 51%). These proportions were different than the expected values (25%, 50%, 25%, chi-square = 8.02, df = 2, p =0.0181) 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 three drug combination of meperidine, midazolam, hydroxyzine was used in 57% of the cases. The second most common drug regimen was meperidine, vistiril, and chloral hydrate, used in 31% of the cases. The meperidine and midazolam combination was used three times and the meperidine and hydroxyzine combination was used once. In one case, Propofol® was used by converting to an intravenous sedation (IVS) due to failed oral sedation.

In the study cohort (n=49), 49% received restorations (n = 24), 19% extractions (n = 9), 20% both restorations and extractions (n = 10), and in 12% of the cases the planned procedures were not performed and the process was aborted (n = 6). There were no instances of apnea or nausea, two instances of vomiting, three 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 North Carolina Behavior Rating Scale Scores and Overall Effectiveness of Sedation Scores between three genotype groups: CYP2B6*1*1, CYP2B6*1*6 and CYP2B6*6*6 during oral sedation. The NCBRS was recorded on 363 occasions (between 0 and 14 times per patient) and had a mean = 1.87, SD = 1.06. NCBRS was not assessed during the post-operative period. Table 3 shows the Analysis of NCBRS during the oral sedations. The results of the repeated-measures ANOVA indicated that NCBRS did vary across event types ($p < 0.0001$), that the genotypes did not differ ($p = 0.0897$) within themselves and that the event differences did not vary within the genotype ($p > 0.2$, see Table 4). However, within the Intraoperative measures, the three genotypes were significantly different ($p = 0.0221$). Specifically, the 1*1 homozygous wild type's intraoperative behavior was significantly different than the 6*6 homozygous variant's ($p = 0.0061$) (see Table 3 and Figure 2).

Table 4 shows the number of individuals in each genotype and sedation effectiveness combination. The genotype groups did not show a statistically significant difference in the overall effectiveness (Wilcoxon rank-sum chi-square = 5.68, df = 2, $p = 0.0585$), but a strong trend towards significance. The overall effectiveness of sedation score may be high for some

patients. If the patient was extremely vocal during the procedure, the treating dentist may have rated the overall sedation in a more negative manner despite the fact the child remained still and treatment proceeded without complications. However, as may be seen in the table 4, 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 Versed ($p = 0.0878$), Chloral Hydrate ($p = 0.0140$) and Propofol ($p = 0.0088$) emerged as potential confounders (using an alpha cut-off of 0.2). There is some indication of a race difference ($p = 0.0741$). Including these in the model did not change the conclusion that overall effectiveness scores did not differ by genotype ($p = 0.2980$).

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: 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 heart rate, (HR) was recorded on 581 occasions (between 2 and 19 times per patient) and had a mean = 100.4, SD = 19.9. The results of the repeated-measures ANOVA indicated that HR did vary across event types ($p < .0001$), that the genotypes did not differ ($p > 0.4$) and that the event differences did not vary within genotype ($p > 0.4$, see Table 6). The estimated mean heart rate for each event is also shown in Table 6 and Figure 3. 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 555 occasions (between 2 and 18 times per patient) and had a mean = 119.7, SD = 19.3. The results of the repeated-measures ANOVA indicated that Sys-BP did vary across event types ($p = .0014$), that the genotypes did not differ ($p > 0.7$) and that the event differences did not vary within genotype ($p > 0.5$, see Table 7). The estimated mean systolic BP for each event is also shown in Table 7 and Figure 4. Tukey's HSD indicated that only the IntraOp value was significantly higher than PreOp and Baseline.

The diastolic blood pressure (Dia-BP) was recorded on 555 occasions (between 2 and 18 times per patient) and had a mean = 67.0, SD = 12.8. The results of the repeated-measures ANOVA indicated that Dia-BP did vary across event types ($p = .0034$), that the genotypes did not differ ($p > 0.9$) but the event differences did vary with genotype ($p = 0.0275$, see Table 5). The estimated mean dia-BP for each event is also shown in Table 8 and Figure 5. Overall, Tukey's HSD indicated that the PostOp values were higher than Baseline or PreOp and other differences were not significant. But, the significant interaction indicates that this pattern may be different depending upon the genotype. Specifically, there is no difference between the event types within the 1*1 genotype group ($p > 0.5$).

The oxygen saturation, SaO_2, was recorded on 577 occasions (between 2 and 19 times per patient) and had a mean = 98.7, 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 vary across event types ($p > 0.0497$), that the genotypes did not differ ($p > 0.8$) and that

the event differences did not vary within genotype ($p > .9$, see Table 9). The estimated mean SaO_2 for each event is also shown in Table 9 and Figure 6. There were three cases where patients experienced desaturation (<95% SaO_2).

DISCUSSION

Genetic findings:

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 heterozygous had EFV concentration >4μg/mL, which is the toxic level. Nearly 20% of homozygous 1*1 and 2% of homozygous 6*6 showed sub therapeutic level of EVF of <1μg/mL.[28] The clinical relevance to their research was the individuals who carried the wild type allele had sub therapeutic 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 10%, 39% and 51% of our population, respectively. There were no statistical significant differences found between the demographic characteristics and the genotypes (ps > 0.09).

Behavioral Findings

Interestingly, our study results showed the opposite of what was expected based on *in vitro* studies of CYP2B6 function. There was a statistical significant difference in NCBRS scores and genotypes during the intraoperative phases of oral sedation ($p = 0.0221$) (see Table 3). Specifically, the homozygous wild-type CYP2B6*1 was significantly different than the homozygous variant CYP2B6*6 as can be seen by observing the 95% CI ($p = 0.0061$) (see Table 3 and Figure 2). The CYP2B6*1 showed significantly more favorable NCBRS scores than the CYP2B6?*6 during the intraoperative treatment intervals. This finding is of significant importance to practitioners in that the success of sedation often depends on behavior at the time of dental treatment or intraoperative phase.

There was not a statistical significance in OESS outcome, (chi-square = 5.68, df = 2, $p = 0.0585$) between the genotypes, but a strong trend towards significance. Table 4 showed that the homozygous wild-type, 1*1, had a median OESS 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 median 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*1 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 auto activation 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 4 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. One patient with the variant allele had an NCBRS and OESS of 1. This patient differed from the other 6*6 patients in that Chloral hydrate was used in the drug regimen.

It appears that CYP2B6 and its variants activity may 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 auto activation.

Physiologic findings

In the study population (n=49), 12% were aborted due to the patient's behaviors. Adverse events were reported as followed: 3 cases of excessive sedation (>30 minutes for recovery), 2 case of vomiting, and 3 cases of desaturation. There were no instances of apnea or nausea. In pediatric patients, nausea does not always precede 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 three 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 and systolic blood pressure 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, 105.83 beats/minute during the oral sedation fell within the normal range for children age 3 – 5, which is 80-125 beats/minute. In addition, the average systolic pressure was 121.18 during the intraoperative phase, which is on

the high end of normal limits of systolic pressure for children age 3 – 5, which is 108-121 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. Our research allows for the conclusion that variations of the CYP2B6 enzyme could predict for successful behavior in oral sedations using meperidine. 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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Literature Cited

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Table 1: Demographic Characteristics

Characteristic	n	%
Race		
AA	32	65
Asian	1	2
C	12	24
O	4	8
Ethnicity		
B	1	2
H	2	4
N	45	92
Y	1	2
Gender		
F	28	57
M	21	43
ASA (1,2)		
1	39	80
2	10	20
Wt (kg)		
Mean	22.0	
SD	5.5	
Min.	15	
Max	38	
Duration of tx (min)		
Mean	24.8	
SD	13.5	
Min.	5	
Max	63	
Age		
Mean	4.8	
SD	1.1	
Min.	3	
Max	8	
Age (months)		
Mean	63.1	
SD	12.9	
Min.	41	
Max	101	

Table 2: Medications

Medications	n	%
Drug regime		
Dem Vis Ver	28	57
Dem Vis CH	15	31
Dem Ver	3	6
Dem Vis	1	2
Dem Vis Ver CH Pro	1	2
Dem Vis Ver Pro	1	2
Local		
none	8	16
L+epi	19	39
L+epi Sept	3	6
Sept	19	39
N2O use		
N2O	42	86
none	7	14

Abbreviations: Dem = Demerol, Vis = Vistaril, Ver = Versed, CH = Chloral hydrate, Pro = Propofol, L = Lidocaine, Sept = Septocaine, N2O = Nitrous oxide.

Table 3: Analysis of NCBRS

Source	df Num.	df Den.	F	p-value
Event	2	308.9	18.42	<.0001
Genotype	2	52.99	2.524	0.0897
Event*Genotype	4	308.3	1.343	0.2540

Event	LS Mean	95% CI	
Genotype 1*1			
Baseline	1.47	1.00	1.95
PreOp	1.49	1.02	1.96
IntraOp	1.91	1.55	2.28
Genotype 1*6			
Baseline	1.26	0.83	1.69
PreOp	1.44	1.02	1.87
IntraOp	2.20	1.87	2.52
Genotype 6*6			
Baseline	2.00	1.07	2.93
PreOp	2.40	1.47	3.33
IntraOp	3.10	2.35	3.84

Table 4: Comparing Overall Effectiveness

CYP2B6	Overall Effectiveness				
genotype	1	2	3	4	Median
1*1	7	5	6	1	2
1*6	5	6	5	9	3
6*6	1		1	3	4

Table 5: Analysis of Heart Rate

Source	df Num.	df Den.	F	p-value
Event	3	530.40	14.51	<.0001
Genotype	2	51.63	0.77	0.4686
Event*Genotype	6	529.80	1.11	0.3568

Event	LS Mean	SE	95% CI	
Baseline	89.70	3.49	82.80	96.61
PreOp	94.86	3.65	87.65	102.08
IntraOp	105.83	2.77	100.29	111.37
PostOp	100.84	2.79	95.25	106.43

Table 6: Analysis of Systolic Blood Pressure

Source	df Num.	df Den.	F	p-value
Event	3	503.30	5.27	0.0014
Genotype	2	49.84	0.30	0.7448
Event*Genotype	6	502.80	0.82	0.5564

Event	LS Mean	SE	95% CI	
Baseline	113.61	3.75	106.20	121.03
PreOp	112.55	3.54	105.55	119.55
IntraOp	121.18	2.89	115.38	126.97
PostOp	117.78	2.93	111.91	123.65

Table 7: Analysis of Diastolic Blood Pressure

Source	df Num.	df Den.	F	p-value
Event	3	508.20	4.62	0.0034
Genotype	2	55.48	0.04	0.9590
Event*Genotype	6	507.80	2.39	0.0275

Event	LS Mean	SE	95% CI	
Baseline	63.80	2.45	58.97	68.63
PreOp	63.21	2.27	58.72	67.69
IntraOp	68.05	1.67	64.70	71.39
PostOp	69.38	1.71	65.97	72.79

Table 8: Analysis of Oxygen Saturation

Source	df Num.	df Den.	F	p-value
Event	3	530.40	2.63	0.0497
Genotype	2	56.68	0.19	0.8265
Event*Genotype	6	530.40	0.25	0.9599

Event	LS Mean	95% CI	
Baseline	98.78	98.27	99.30
PreOp	98.46	97.93	99.00
IntraOp	98.49	98.14	98.85
PostOp	98.87	98.51	99.23

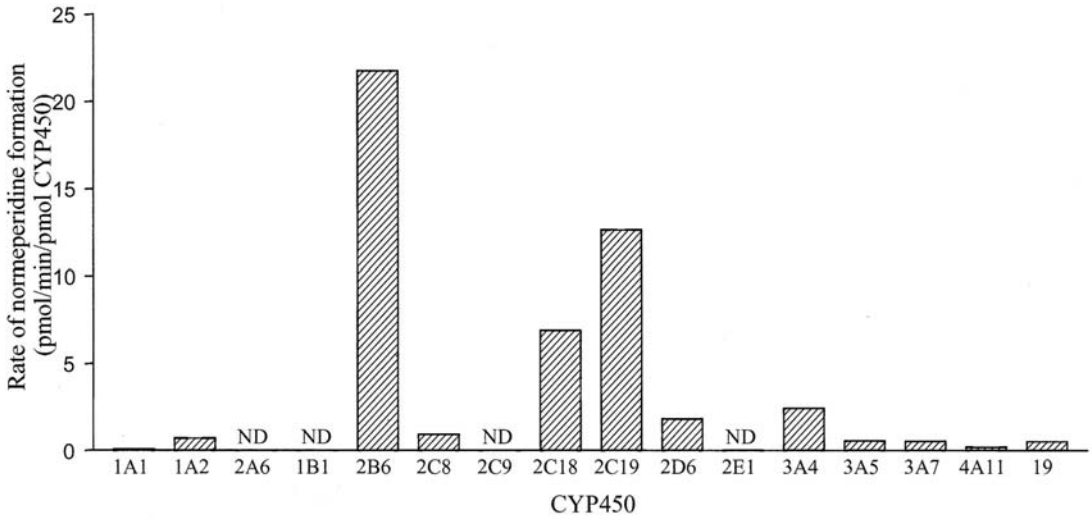

Figure 1. CYP450 isozymes responsible for meperidine metabolism. As illustrated in the figure, it has been demonstrated *in vitro* that Cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6) is the enzyme primarily responsible for metabolism of meperidine [14]

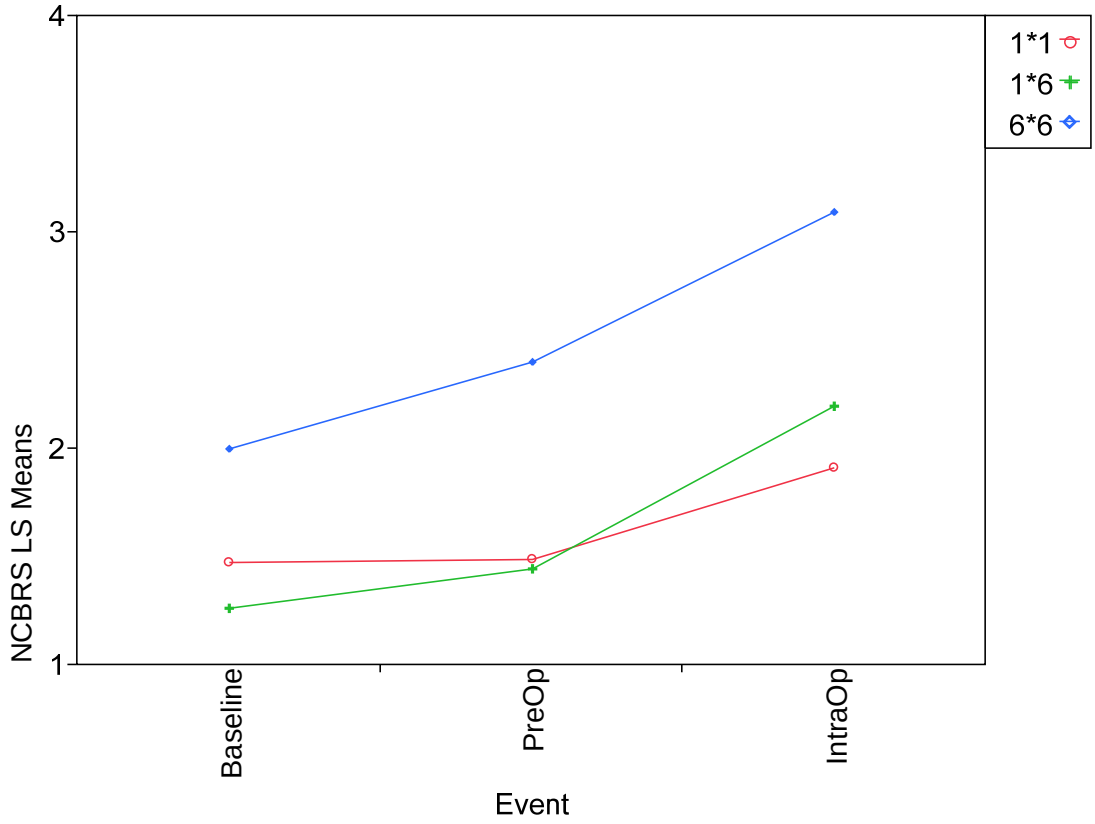

Figure 2: NCBRS

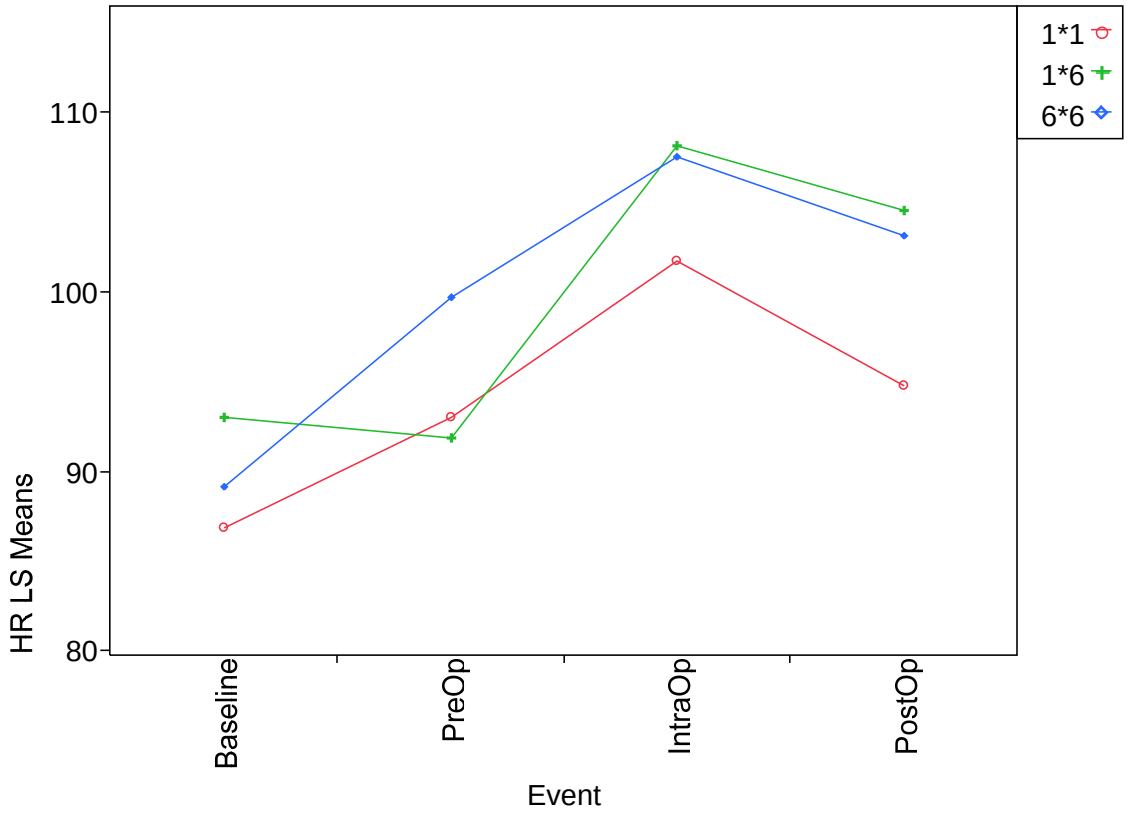

Figure 3: Analysis of Heart Rate

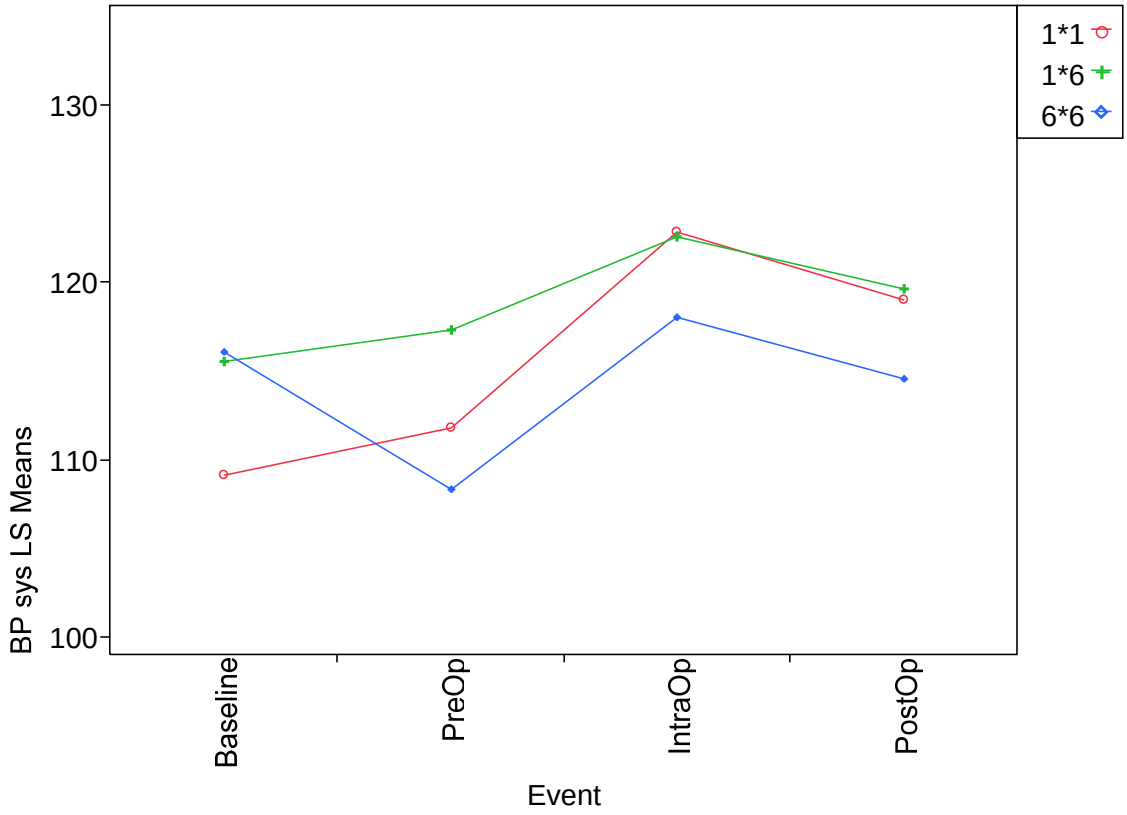

Figure 4: Systolic Blood Pressure

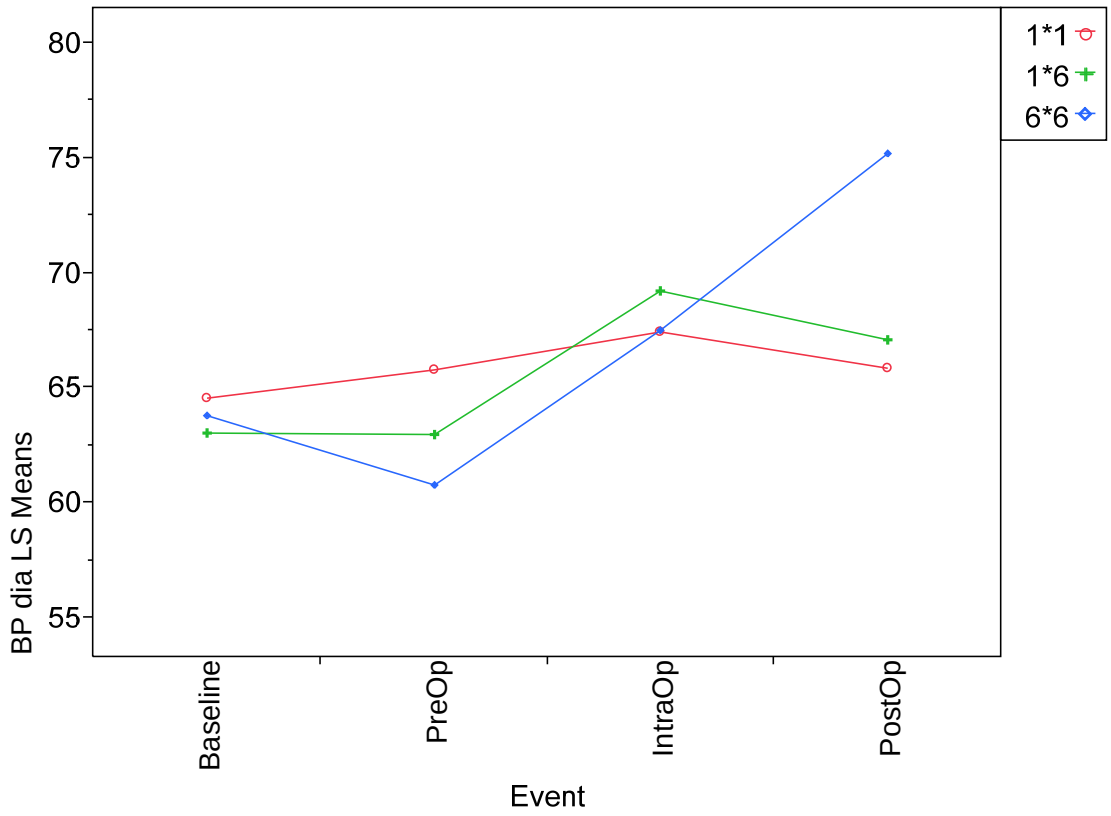

Figure 5: Diastolic Blood Pressure

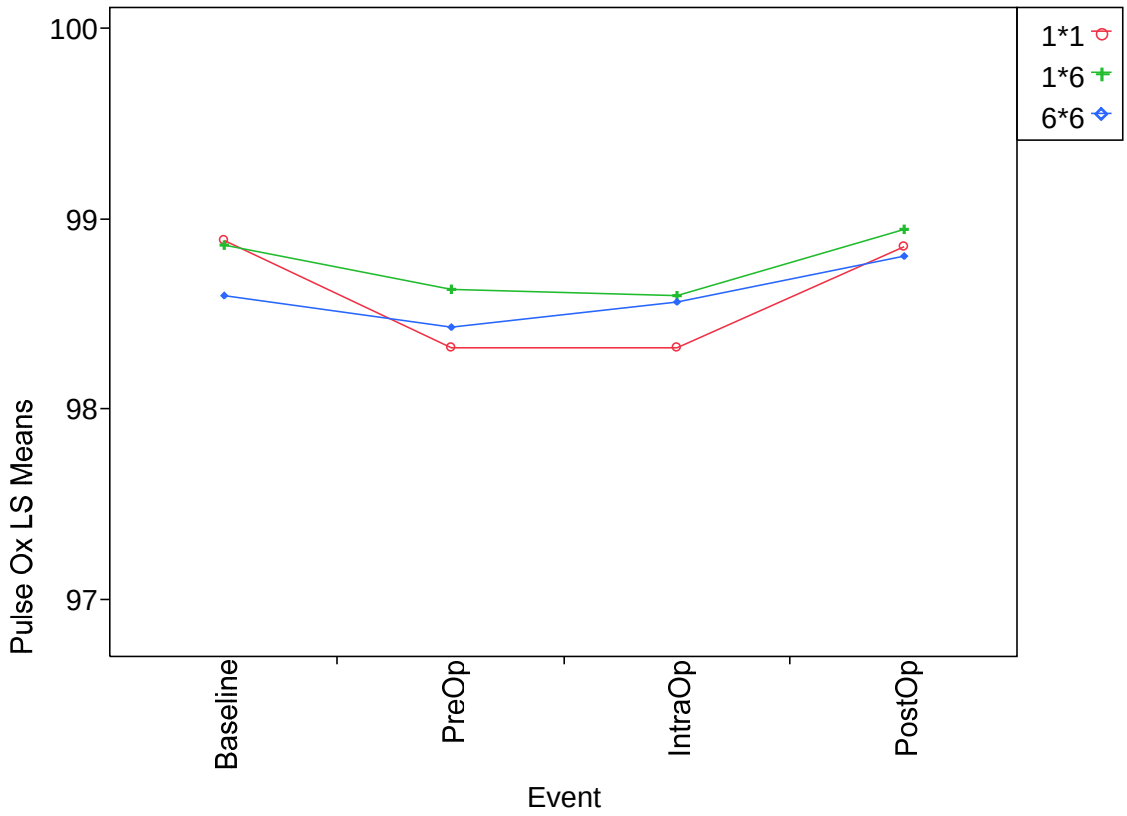

Figure 6: Pulse Oxygen

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

Heath Austin Whitfield was born on May 19th, 1981 in Weatherford, Oklahoma. He graduated from Weatherford High School, Weatherford, OK in 1999. He went on to attend Southwestern Oklahoma State University, Weatherford, OK, on a full academic scholarship studying Health Science and Chemistry. He then attended The University of Oklahoma College of Dentistry, graduating with *distinction* and *honors,* where he obtained the degree of Doctor of Dental Surgery in 2008.