The Prevalence of Ultrarapid Metabolizers of Codeine in a Diverse Urban Population

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I n August 2012, the US Food and Drug Administration (FDA) released a safety report regarding the use of codeine in children who had undergone adenotonsillectomy for obstructive sleep apnea. The report describes “three pediatric deaths and one non-fatal but life-threatening case of respiratory depression . . . documented in the medical literature.”1 These 4 children, ages 2 to 5 years, were found to possess multiple copies of a fully functional CYP2D6 allele that resulted in the ultrarapid metabolism of codeine. In February 2013, the FDA required a black box warning be placed on codeine and declared adenoidectomy or tonsillectomy a contraindication to the use of codeine in children.2

The cytochrome p450 enzyme, CYP2D6, is the primary agent of codeine metabolism. Through the action of this hepatic enzyme, codeine is broken down into multiple active by-products, including morphine, which is 10 times more potent than codeine. Consequently, ultrarapid codeine metabolism profoundly increases its analgesic impact and the potential for adverse side effects, including respiratory depression.3 Conversely, poor metabolizers will not generate any of codeine’s more potent by-products, undermining its role as an analgesic. In 1 recent study, as many as 35% of children had no morphine in their serum after administration of codeine.4

While the dangers of ultrarapid metabolism of codeine are well documented, other commonly prescribed opiates, including hydrocodone and oxycodone, are similarly metabolized to pharmacologically active by-products by the CYP2D6 enzyme. Hydrocodone generates hydromorphone, which binds to \( \mu \)-opioid receptors with an affinity 10 to 33 times stronger than its parent drug. Approximately 11% of

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oxycodone is metabolized by CYP2D6 to oxymorphone, a compound that is 8 times more potent than oxycodone. Many clinicians have followed the FDA’s warning to avoid using codeine after adenotonsillectomy. However, in many cases, codeine has been replaced by hydrocodone or oxycodone, which may also pose a risk to patients who are CYP2D6 ultrarapid metabolizers.

CYP2D6 comprises approximately 2% to 5% of the cytochrome P450 enzymes, but it metabolizes a disproportionately large percentage of pharmaceuticals (about 25%). Among the diverse array of drugs metabolized by the CYP2D6 enzyme are many selective serotonin reuptake inhibitors, tricyclic antidepressants, antipsychotics, opioids, and common medicines such as diltiazem, metoprolol, and warfarin. More than 100 DNA sequence variants of this gene have been identified. The CYP2D6 gene carries several known polymorphisms that significantly alter enzyme function and create wide variability in the analgesic effects of codeine between individuals. Genotypes have been grouped into 4 phenotypic categories based on their observed rate of metabolism of target drugs: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UMs). Individuals who are UMs have multiple copies of a fully functional CYP2D6 allele, resulting in elevated levels of messenger RNA (mRNA) and active enzyme. The relationship between the number of active copies of the CYP2D6 gene in UM individuals and the rate of codeine clearance was nearly linear in a recent study. The same study attributed 63% of the variability in morphine metabolism to the CYP2D6 genotype.

The incidence of the various CYP2D6 phenotypes in the United States is unknown and is made increasingly unclear by the wide variability of CYP2D6 alleles among populations with different ancestral origins. Estimates of the prevalence of UMs range from 2% in a review of multiple populations within the United States to 3% in a Northern European cohort to 40% in a North African population. A 2006 study of 222 African Americans revealed that 4.5% were UMs. The wide variability in rates of CYP2D6 polymorphisms, the relative dearth of data from American cohorts, and the tremendous ethnic diversity found in US cities all confound researchers and clinicians attempting to estimate UM prevalence in the US population. Consequently, the clinical utility of routine genotyping for CYP2D6 is uncertain.

Identifying the risks associated with the UM phenotype is challenging in the United States, a country comprising primarily immigrants, and also daunting in the ethnically diverse populations in many US cities. On the most recent census, the majority of the population of Bronx county, New York, identified as Hispanic or Latino (53.8%). Among these, 21.8% self-identify as Puerto Rican, 5% as Mexican, and 26.4% as “other Hispanic.” A total of 36.2% of Bronx residents self-identify as African American or black. Only 4% of Bronx residents identify as Asian, and 24.6% identify as Caucasian or white. The majority Hispanic population increases the uncertainty, due to the limited literature regarding distribution of CYP2D6 polymorphisms in Hispanic populations. We are unaware of any estimates of UM prevalence in either Puerto Rican or Dominican populations.

Pharmacogenomics is the study of the role of an individual’s genetics in the metabolism and response to drugs, and its application in clinical medicine has grown dramatically in recent years. As our understanding of the human genome continues to evolve and costs of genomic analysis decrease, a pharmacogenomic approach to providing care is increasingly feasible. Defining the incidence of the CYP2D6 UM phenotype is the first step toward assessing the utility of this approach to drug selection and dosing. Our study describes the results of CYP2D6 genotyping on DNA specimens collected from children in the Bronx. We examine the distribution of CYP2D6 polymorphisms among several racial and ethnic groups represented in our diverse health care community. This information may provide insights that allow us to optimize the safety and efficacy of opiates and other drugs commonly metabolized by CYP2D6.

Methods
Patient Cohort

The analysis was conducted on previously banked DNA samples from 256 children with nonsyndromic sensorineural hearing loss. The families of these patients had consented for genetic analysis through a separate institutional review board (IRB)-approved protocol, which included broad consent for further study of the banked samples. The current study was approved by the IRB at the Albert Einstein College of Medicine.

Each participant or family member self-reported his or her ethnic background. Much of the published data regarding the prevalence of various CYP2D6 phenotypes have been reported for geographic regions as opposed to ethnic identity. Accordingly, in addition to racial and ethnic information, the country in which the participants’ grandparents were born was also recorded. This information served as a surrogate for the child’s country of ancestral origin. Those who identified as multiethnic or who noted grandparents from multiple countries were tallied in each relevant subpopulation. While this information lacks the precision often provided by genetic ancestry testing, self-reported country of origin is readily available to clinicians and may be valuable in screening for children at elevated risk for ultrarapid metabolism of CYP2D6-metabolized pharmaceuticals.

Sequenom iPlex CYP2D6 Assay

The specimens were analyzed using the Sequenom iPlex CYP2D6 (Sequenom, San Diego, California) assay. This high-throughput, multiplex assay is designed to identify 63 single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs) that affect the function and expression level of the CYP2D6 enzyme.

Bioinformatics

Each of the tested CYP2D6 SNPs has been characterized in terms of the rate at which it metabolizes its substrate.
Specific SNPs code for an enzyme with full activity, limited activity, or no activity. Based on the diplotype and CNV, CYP2D6 SNPs and CNVs were grouped into phenotypic categories that represent the rate at which they would be expected to metabolize substrates of the CYP2D6 enzyme. A fully functional allele is given an activity score of 1.0, a reduced function allele is scored 0.5, and a nonfunctional allele is assigned a score of 0. The sum of activity scores is used to predict phenotype (UM, EM, IM, or PM) (Table 1). Those subjects with duplicate copies of fully active alleles are categorized as UMs.

Statistical Analysis
The primary objective of the study was to establish an estimate of the incidence of UMs in the Bronx population. A secondary goal was to describe the distribution of metabolic phenotypes across racial and ethnic subgroups. A power calculation was performed to approximate the true rate of UMs with a deviation of less than 3% and a confidence interval of 95%. A target sample size of 203 patients was calculated assuming a binomial distribution with an estimated UM rate in our population of 5%. Subsequent analysis compared rates of UM in ethnic or geographic subgroups against the rates identified in the entire test cohort. Most proportions were compared using $\chi^2$ analyses to determine statistically significant differences. Fisher exact tests were applied where appropriate. To compare the identified rates of UM individuals in the study population to the Bronx population, a 1-sample proportions test was used. All analyses were carried out using Stata SE version 14.2 (StataCorp, College Station, Texas) and GraphPad Prism version 7.03 (GraphPad Software, La Jolla, California).

Results
The racial/ethnic distribution of the study cohort was similar to that of the community, with the following specific differences: the Hispanic/Latino distribution was similar (Bronx 54.6% vs 45.8% cohort, $P = .07$), but the study cohort was made up of a significantly lower proportion of African Americans (Bronx 43.3% vs 18% cohort, $P < .0001$), while the proportion of Caucasians was significantly higher (Bronx 10.5% vs 31.5% cohort, $P < .0001$) (Figure 1). The most frequently reported countries of origin were Caribbean or North American countries, with the most common being the United States (34.1%), Puerto Rico (15.8%), and the Dominican Republic (14.2%).

Among the 256 specimens assayed, 191 yielded a predictable phenotype. Eighteen of 191 children (9.42%) were identified as UMs. Most participants in our cohort (154/191 children, 80.6%) were found to be EMs. Sixteen children (8.37%) were IMs. Only 3 of 191 children in our cohort (1.57%) were PMs (Figure 2). The proportions of UMs, EMs, IMs, and PMs were generally statistically significantly different when compared across all 3 race-ethnicities ($\chi^2 = 13.77$, df = 6, $P = .03$; Figure 3). Since the primary category of interest was the UM group, the proportion of this subgroup compared to the other phenotypes was compared across race-ethnicity groups. The incidence of UMs was determined among each of several demographic groups, including those who self-identified as Caucasian, African American, or Hispanic. Rates of UMs in Caucasian and Hispanic patients were 11.3% and 11.2%, respectively, while UMs in African American participants included 3.6%; however, no differences observed between race-ethnicity groups reached statistical significance.

In addition, we looked at those participants whose country of ancestral origin was the United States, a Caribbean nation (Puerto Rico, Dominican Republic, Cuba, Haiti,

### Table 1: Phenotype Predicted by the Sum of Haplotypes’ Activity Scores.

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<thead>
<tr>
<th>Predicted Phenotype</th>
<th>Activity Score</th>
<th>Haplotypes</th>
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<tbody>
<tr>
<td>Poor metabolizer</td>
<td>0</td>
<td>Two nonfunctional alleles</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>0.5</td>
<td>One nonfunctional and 1 reduced functional allele</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td>1.0-2.0</td>
<td>One fully functional allele in combination with any other OR 2 intermediate function alleles</td>
</tr>
<tr>
<td>Ultrarapid metabolizer</td>
<td>&gt;2.0</td>
<td>Multiple copies of a fully functional allele</td>
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Jamaica, and Antigua), Latin America, or Central America (Figure 4). Rates of UMs in patients with ancestral origin in the United States were 6.25%, while rates of UMs in patients from the Caribbean, South America, and Central America were 5 of 61 (8.2%), 0 of 10 (0%), and 5 of 16 (31.3%), respectively. Subgroup analysis identified a statistically significant increase in the number of UM subjects from Central America compared with the rates in our study population ($P = .008$). The difference in rates of UMs was not significant in every other analyzed subgroup.

Sixty-five of our initial 256 samples yielded a genotype without a predictable CYP2D6 phenotype. These samples identified an uncharacterized polymorphism or revealed a haplotype that did not correspond with a predictable phenotype (Figure 5). In 22 cases (8.6%), the assays did not provide any information. A further 9 samples (3.5%) identified a novel or previously uncharacterized haplotype, and no prediction could be made regarding the associated phenotype. In 5 cases (1.9%), the number of copies of the identified haplotype could not be determined and the result would
have differentiated between an EM or UM phenotype. A further 11 assays (4.3%) could not distinguish between at least 2 possible alleles of varying functionality. Finally, 18 samples (7.0%) revealed an allelic duplication; however, either a fully functional or a reduced function allele may have been present in multiple copies. In these 18 cases, the resulting phenotype would have been either EM or UM. The ethnicities of patients represented in these inconclusive assays are statistically similar to the test population (26.2% Caucasian, 40% Hispanic, 26.2% black, and 6.2% Asian), \( P = .14 \) by \( \chi^2 \) analysis. This suggests that bias was not introduced by the unresolved assays (see Supplemental Table S1 in the online version of the article).

**Discussion**

This study is the first to characterize the rate of the CYP2D6 UM phenotype in an ethnically heterogeneous US population. As this enzyme metabolizes as much as 25% of pharmaceuticals, including many opiates, establishing a rate of the UM phenotype is an important early step in optimizing the safety and effectiveness of these drugs. This study represents an initial step in establishing the utility of a pharmacogenomics approach to drug prescription in a diverse US population.

Most subjects with an identified phenotype (154/191, 80.6%) were EMs, while only 1.6% (3/191) were PMs. This suggests that most patients in the tested population would readily metabolize codeine or hydrocodone to their more potent metabolites, and both would provide effective analgesia in these patients. The prevalence of UMs (9.42%) in our study is higher than previously published data on a US population that had estimated the prevalence between 2% and 4%.\(^9\)\(^,\)\(^10\) This elevated and clinically significant portion of UMs in our study population is at risk for adverse respiratory events in response to certain opiates.

The distribution of race, ethnicity, and country of ancestral origin found in the study sample was reflective of the Bronx community. Our study population was tremendously diverse, although a large minority of our participants (70 children, 34.1%) identified the United States as the birthplace of at least 1 grandparent. This subcohort of US children was itself heterogeneous. Only 1 subject identified as American Indian, and 28 of 70 patients from the United States self-identified as black or Hispanic/Latino. These findings call into question the utility of much of the published literature on rates of CYP2D6 phenotypes, which often narrowly focuses on homogeneous populations from a particular geographic region.

Perhaps the most remarkable finding in our study is the extraordinary variability in the rates of UMs among the Hispanic participants. The UM rate ranged from 0% (0/10) in South American patients to 8.2% (5/61) in the large Caribbean population to an astounding 31.3% (5/16) in our Central American population. This finding draws attention to the limited or absent data regarding rates of CYP2D6 CNVs in Hispanic and particularly Caribbean and Central American populations, a concern also raised in 2 recent reviews.\(^12\)\(^,\)\(^13\)

The evolving field of pharmacogenomics allows clinicians to tailor drug selection for an individual patient based on genetic determinants of his or her response to a drug. The therapeutic goal is to maximize drug efficacy while minimizing adverse effects.\(^15\)\(^,\)\(^16\) Pharmacogenomics has shown promise in many fields. Evidence suggests a role for dosing many cardiovascular drugs such as warfarin, clopidogrel, and statins based on specific haplotypes.\(^17\) Recent studies suggest that pharmacogenomics may play a role in limiting toxicity resulting from breast cancer chemotherapy. Polymorphisms of the gene encoding methylene tetrahydrofolate reductase (MTHFR), an enzyme critical to the metabolism of folate, have been shown to affect the cytotoxic effects of 5-fluorouracil (5-FU) and methotrexate in patients with breast cancer.\(^18\) Another investigation of breast cancer chemotherapeutic agents identified genes that may influence the efficacy of doxorubicin and cyclophosphamide and the toxicity of tamoxifen.\(^19\) There is also evidence that CYP2D6 polymorphisms modulate the efficacy of tamoxifen.\(^20\)

Due to the extraordinary variability of the sequence and structure of the CYP2D6 gene and the reports of postoperative mortality associated with certain polymorphisms, it is imperative that clinicians have substantive data regarding the frequency of the CYP2D6 haplotypes to develop a safe strategy for administering postoperative codeine or other similarly metabolized opiates. PM patients will obtain no analgesic benefit from codeine, limiting its utility in populations with a high incidence of PM incidence. Conversely, use of codeine and other opiates in populations with a high incidence of UMs may put an unacceptably high number of patients at risk for adverse events.

Our study has several limitations. First, our study cohort was a convenience sample comprising children with congenital sensorineural hearing loss, which may not reflect a random sampling of the population we were targeting. We feel that any genetic aberrations related to this cohort’s hearing loss are likely to sort independently of the CYP2D6 gene, but that is speculative. A second limitation is the failure of 25.4% of our assays to identify a predicted CYP2D6 phenotype. Although we see no reason that these failed assays would be disproportionately associated with any particular CYP2D6 phenotype, these indeterminate assays introduce uncertainty into our findings. Finally, we tested 256 participants to meet or exceed the sample size of 203 determined by our initial power analysis. Due to the number of uninterpretable assays, we obtained information on only 191 children. The ethnic distribution among the uninterpretable assays mirrored those of the successful assays, so this limitation is unlikely to affect our analyses. Despite this technical challenge, we were able to identify differences in the incidence of UMs between various subpopulations, suggesting a role for pharmacogenomics in the administration of opiates.

**Conclusion**

Our data demonstrate that CYP2D6 metabolism varies widely across ethnic groups and geographic subpopulations. Metabolism of codeine and other drugs may be highly
variable in racially and ethnically diverse populations. This work supports utility of a pharmacogenomic approach to opiate prescribing among populations where rates of UMs are suspected to be high or are unknown.

**Author Contributions**

Jordan Virbalas, conception, study design, data acquisition, data analysis, manuscript writing; Bernice E. Morrow, study design, manuscript revision; David Reynolds, data acquisition, manuscript revision; John P. Bent, study design, manuscript revision; Thomas J. Ow, conception, study design, manuscript development and revision.

**Disclosures**

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**Supplemental Material**

Additional supporting information is available in the online version of the article.

**References**


