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Research Article

Pharmacogenetic Analysis of Three Cytochrome P450 Gene Polymorphisms (CYP2D6, CYP3A4, and CYP3A5) in Methadone Maintenance Patients

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Abstract

Best practice guidelines and the Substance Abuse and Mental Health Services Administration (SAMHSA) Treatment Improvement Protocol 63 mandates methadone maintenance induction with low initial dosage and a small, slow increase to avoid overdose and to reach a steady state. Primarily metabolized by the cytochrome 2D6 enzyme, it is shown that polymorphisms in this subunit can greatly affect the rate at which methadone is metabolized in individuals. Six genetic polymorphisms are commonly identified which ranged from poor metabolizers to ultrarapid metabolizers. Patients who can't reach a steady state dose in a reasonable time because of slow incremental dose increases can be more susceptible to relapse and slow metabolizers can be susceptible to overdose. Phenotypic data of CYP2D6 polymorphisms can guide better initial dosing for patients that may require a higher dose to reach a steady state. To date, there has been no way to predict the dose needed for a steady state. A total of 25 patients were enrolled and genotyped with an average time on methadone of 14.2 years and ages 31 to 75 years. Ultra-rapid metabolizers were shown to require significantly higher doses of methadone to reach a steady state. This was a pilot study that supports further research into methadone pharmacogenomics.

Keywords: Methadone; Pharmacogenomics; CYP2D6

Introduction

On methadone maintenance for over a decade, a woman in her 30's has been described as stable on 60 mg of daily doses of methadone but occasionally testing negative for methadone on monthly urine toxicology samples. Patient has had multiple complex medical complaints over the years and is seeing her physician(s) for them. She reported that she took multiple herbal supplements and was drinking large amounts of fluids for her medical complaints.

The patient's lifestyle was very stable with no history of criminal justice or other illegal activities. She never complained of being in withdrawal nor did she show withdrawal signs and was seen as a "model" patient.

Due to the negative urine test results, however, the staff suspected and accused her of diverting her methadone doses and threatened take home reductions or possible discharge. The pa-

tient became very angry and any therapeutic alliance with her was negatively affected.

Due to the patient's vehement protests of not diverting, the clinic arranged for onsite observation of dosing and collection of urine samples for toxicology testing at intervals over most of the day. The patient was found to be a rapid metabolizer and all suspicion and accusations were dropped.

In this case, testing upon initial induction of methadone would have avoided this individual being subject to further stigmatization related to society's general image of "addict" and "liar" and might have urged the clinic to look further into the patient's medical issues to assist her in resolving or alleviating them. This shed light on the consequences of inadequate dosing.

The opioid epidemic has grown into a prominent public health issue. Abuse of opioids can lead to addiction, severe respiratory depression and death. 2015-2017 saw significant increases in synthetic opioid deaths in all racial and ethnic demographics as illicitly manufactured fentanyl continues to fuel the US opioid epidemic[1]. Reducing physical dependence involves confronting withdrawal symptoms that include diarrhea, vomiting, nausea, agitation, anxiety and increased lacrimation which itself can impair daily life and, if not monitored correctly, can result in death due to dehydration or hypernatremia. Relapse after a period of detox can be even more lethal as patients' tolerance decreases and they cannot handle previously tolerated larger doses.

Methadone is an opioid with a long half-life that allows daily dosing and can treat moderate to severe pain. When used together with medical supervision and counseling, it can be an effective treatment for opioid use disorder. Over the last decade, the use of methadone for opioid dependence has grown substantially. Opioid prescriptions by physicians rose significantly from 2007-2010 but leveled off in the early 2010's [1]. However, synthetic opioids have been an increasing factor in opioid related deaths. From 2015-2017 the most significant increases in synthetic opioid deaths were in blacks aged 45-64 years and non-Hispanic whites aged 25-34 years, which increased more than two-fold, 19.3 to 41.9, 21.8 to 42.7, 36.9 to 58.3 in 100,000 respectively [1].

Methadone maintenance has been utilized and evaluated since its development in 1964 as a medical response to the post-World War II heroin epidemic in New York City. The findings of major early studies have been consistent. Methadone maintenance reduces and eliminates the use of heroin, reduces the death rates and criminality associated with heroin use, and allows patients to improve their health and social productivity. In addition, enrollment in methadone maintenance has the potential to reduce the transmission of infectious diseases associated with heroin injection, such as hepatitis and HIV. The principal effects of methadone maintenance are to relieve narcotic craving, suppress the abstinence syndrome, and block the euphoric, habit-forming effects associated with heroin. A majority of patients require 80-120 mg/d of methadone, or more, to achieve these effects and require treatment for an indefinite period of time, since methadone maintenance is a corrective but not a curative treatment for heroin addiction. Lower doses may not be as effective or provide the blockade effect. Providing inadequate doses of methadone has shown to increase the rate of relapse for patients on methadone maintenance and exemplifies a barrier pharmacogenomics can help bridge [2].

Methadone maintenance has been found to be medically safe and nonsedating. It is also safe for pregnant women battling opioid use disorder. Enzyme activity, specifically in CYP3A4 and CYP2D6 has shown to increase during pregnancy and CYP1A2 shown to decrease due to estrogenic hormones such as estradiol and progesterone [3].

Pharmacogenomics is the growing field that studies the impact that genetic variants have on drug pharmacokinetics and pharmacodynamics for individuals. Pharmacokinetics is what the body does to the drugs, which includes: absorption, distribution, metabolism, and excretion. Pharmacodynamics is the biochemical and physiological effects that the drug has on the body. Pharmacogenomic factors have been increasingly studied for pain treatment as genetic characterizations affect individual responses to drugs which can have safety implications for proper dosing.

The metabolism of methadone occurs via the cytochrome P450 pathway with hepatic N-demethylation to the inactive primary metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine. The cytochrome P450 isoenzymes (CYPs) are a superfamily of enzymes, which are embedded in the phospholipid bilayer of the endoplasmic reticulum. Although the CYPs associated with drug metabolism are mainly found in the liver, they can also be expressed in lower amounts in the gastrointestinal tract, lung, central nervous system, and kidney. Twelve CYPs are thought to be involved in general drug metabolism.

Extensive variations in the expression of specific CYP enzyme levels occur between humans secondary to the presence of genetic polymorphisms and differences in gene regulation. Genetic polymorphisms of cytochrome P450 genes have been described with the existence of poor (zero functional genes), extensive (one or two functional genes), and ultra-rapid metabolizers (three or more functional genes). The CYP2D6 specifically is thought to play a primary role in methadone metabolism. CYP2D6 polymorphisms can lead to different metabolism rates of methadone and therefore can directly affect the dosing needed in order to reach steady state.

Methods

Kū Aloha Ola Mau has offered methadone treatment (Medication Assisted Treatment) since 1976. Its Honolulu program served approximately 150 to 175 patients at the time of this study and offered weekly to monthly group and individual counseling for each patient. Dosing protocols followed Treatment Improvement Protocol 63 SAMHSA Guidelines. The amount and duration of the counseling was dependent on patient progress, program take home status, guidelines and individual need.

After obtaining informed consent, buccal swabs were taken by Kū Aloha Ola Mau volunteers. Buccal swabs are a common, non-invasive way to collect DNA samples for testing from the cells on the inside of a person's cheek. Questionnaires were also collected from the patients. The buccal swabs were labeled with the patient study ID number only and transported in specimen bags to the molecular diagnostics laboratory.

DNA samples from buccal swabs were extracted using a standard ethanol extraction. Detection of single nucleotide polymorphisms were used to identify allele variants in CYP2D6, CYP3A4, and CYP3A5 genes using the QuantStudio 12K™ Flex Real-Time PCR System by ThermoFisher Scientific (Carlsbad, CA). Structural alterations including gene deletion, duplication and rearrangement of CYP2D6 were identified by real-time PCR of CYP2D6 intron 2, intron 6, and exon 9. CYP2D6 copy number changes were analyzed on the ViiA7 real-time multiplex PCR platform by ThermoFisher Scientific (Carlsbad, CA) using known reference sample comparisons. Amplified PCR products were transferred to OpenArray® 384-well sample plates using the QuantStudio™OpenArray®AccuFill™ robot.

Genotyping results were analyzed using QuantStudio™12K Flex Software Results and compared to known genotypic specificities of CYP metabolic groups.

Results

A total of 25 patients were enrolled in this study. Eleven of these patients were women and fourteen were men. The median age at evaluation was 54 years, with ages ranging from 31 to 75 years. Time on methadone ranged from 1 to 40 years. Average time on methadone was 14.2 years. Twelve (48%) reported first developing dependence to prescription opioids while thirteen patients reported first developing dependence to heroin. Daily dosage of methadone ranged from 37 mg to 240 mg. The average dose was 112 mg, while the median dose was 90 mg. 23 (92%) of these patients reported that their current methadone dose was adequate.

Genotypic frequencies ranged from poor metabolizers to ultra-rapid metabolizers in CYP2D6 and CYP2C19. CYP2D6 deletions and duplications were identified. The differences in dosage were clinically significant, but not statistically significant (Kruskal-Wallis test p-value=0.1106). Overall, time on methadone was similar. Using a cutoff of 110 mg QD, the percentage of patients requiring dosages above and below 110 mg was determined in each metabolizer category.

Using the Cochran Armitage test for trend, a statistically significant difference was seen in the proportion of patients requiring less than, and those requiring over 110 mg per day in each metabolizer group. Higher metabolism was associated with dosages above 110 mg.

All Null/Poor metabolizers were below the 110 mg dose. 77.78% of intermediate metabolizers were below the 110 mg dose and 22.2% were above. An equal number of patients with the extensive metabolizer phenotype required less than 100 mg and over 110 mg (60% <100mg and 40% >110mg). Among the ultra-rapid metabolizer phenotype, all patients required QD dosages greater than 110 mg.

While the population size was small, there was an observed difference in the average daily dose of methadone by ethnicity ($p = 0.2133$). Patients self-described as "Asian" had a lower average daily methadone dosage, 80.0 mg. Caucasian and mixed ethnicities ranged from 103.0 and 122.4 mg. Native American and Pacific islanders had the highest average daily dose at 185.0 mg.

Statistical analysis

Statistical methods include using Chi square or Fisher's exact test to analyze categorical data, including methadone dosing, phenotypic expression, and ethnicity, and nonparametric methods to

analyze continuous data. Where appropriate, analysis of variance was used for continuous data. Analysis was performed using SAS 9.4 (SAS Institute, Cary, NC).

Discussion

Reviews issued by the Institute of Medicine and the National Institutes of Health have defined narcotic addiction as a chronic medical disorder and have claimed that methadone maintenance coupled with social services is the most effective treatment for this condition. These agencies recommend reducing governmental regulation to facilitate patient's access to treatment. In addition, they recommend that the number of programs be expanded, and that new models of treatment be implemented, if the nationwide problem of addiction is to be brought under control. The National Institutes of Health also recommend that methadone maintenance be available to persons under legal supervision, such as probationers, parolees and the incarcerated. However, stigma and bias directed at the programs and the patients have hindered expansion and the effective delivery of services. Professional community leadership is necessary to educate the general public if these impediments are to be overcome.

Pharmacogenomic metabolizer differences of the CYP2D6 have shown to support specific dosing recommendations in antidepressants and predicting safety and effectiveness of codeine and oxycodone [4]. Evidence also supports genotype dosing recommendations for antidepressants [5]. According to the Substance Abuse and Mental Health Services Administration guidelines, current methadone maintenance recommendations are to start low and increase gradually over several weeks [6]. A general dosing profile at Kū Aloha Ola Mau is as follows: the maximum starting dose will not exceed 40 mg. Dose increases do not exceed 10 mg at a time with two to three day periods until 60 mg is reached. After 60 mg is reached, there is a holding period for at least a week before a patient can increase any further. Further increases require counselor consent along with patient compliance before a physician approves any dose increase. A patient who may need greater than 180 mg to be on a stable dose can take months until they are able to reach the needed dose. During this time of increase, patients are still feeling the withdrawal effects and can be tempted to continue using heroin or other opiates.

Drs. Vincent Dole and Marie Nyswander, the pioneers of methadone maintenance, found that most patients reached a stable dose between 80-120 mg/day [7]. These findings are supported in the collected data, but also shows outliers which have significantly higher stable doses. Results show a correlation between ultra-rapid metabolizers and higher stable maintenance doses. Genomic information can be helpful in a physician's decision whether to increase a patient's daily dose faster. Clinics can have hundreds of people a day, which means a high case load for each physician. Much of the decision to increase is based on subjective interaction with the patient and reports from the program. Pharmacogenomic data can give an objective analysis of how the body will likely react to the drug. A patient that is found to be an ultra-rapid metabolizer may then be able to increase their doses faster and limit any time where they are feeling adverse withdrawal effects.

There are many other comorbidities and situational factors that also need to be addressed during a patient's treatment. Psychological trauma, legal issues or other restricting factors may need to be addressed but cannot be fully resolved until a stable maintenance dose is reached. Attaining a proper maintenance dose quicker can help to facilitate the resolution of additional problems. Counseling at Kū Aloha Ola Mau is available to patients at every stage of treatment. The clinic sees many different patients dealing with other physical trauma, abusive relationships or homelessness.

Current literature suggests a bell type curve of metabolizers with most ethnicities having significantly more extensive metabolizers and intermediate metabolizers than poor metabolizers and ultra-rapid metabolizers [8-11]. This is also reflected in our data which shows 20 extensive metabolizers and intermediate metabolizers with 3 ultra-rapid metabolizers and 1 poor metabolizer. Native American and Pacific Islander showed the highest maintenance dosing while Asians had the lowest. Our results serve to support a pharmacogenomics correlation between ultra-rapid metabolizer phenotype and significantly higher maintenance dosing and calls for a larger study into the pharmacogenomics of CYP2D6 with methadone.

Limited by a smaller sample size, the patient pool lacked the phenotypic range for CYP3A4 and CYP3A5 to make any significant observations. Other considerations for maintenance dosing include patient weight and concurrent medications. Regardless, the results still have power with using the CYP2D6 enzyme to predict a higher maintenance dose. Pharmacogenomic data for CYP2D6 can also be valuable information in the emergency medicine and operation room settings. It may also have implications in improving treatment for acute and chronic pain. Objective information like this can lend support for more bolder prescriptions.

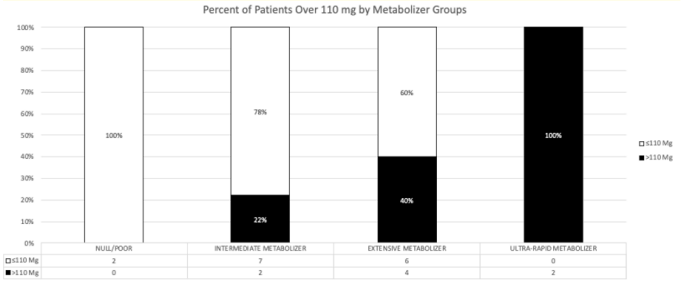

Figure 1: Percent of patients that are over 110 mg of methadone for each metabolizer group.

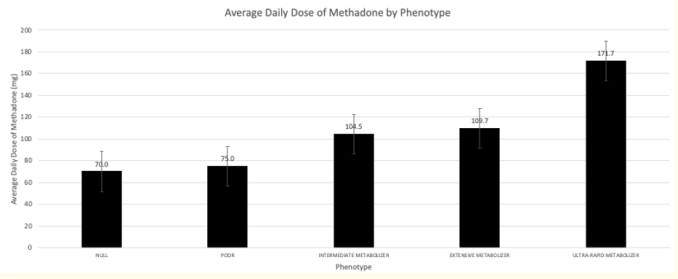

Figure 3: Average daily dose of methadone by ethnicity.

	Null/ poor	Interme-diate metabo-lizer	Extensive metabolizer	Ultra-rapid metabo-lizer	Total
<=110	2 100.00%	7 77.78%	6 60.00%	0 0.00%	15
>110	0 0.00%	2 22.22%	4 40.00%	2 100.00%	8
Total	2	9	10	2	23

Table 1: Percent of patients that are on doses higher or lower doses than 110 mg of methadone for metabolizer groups.

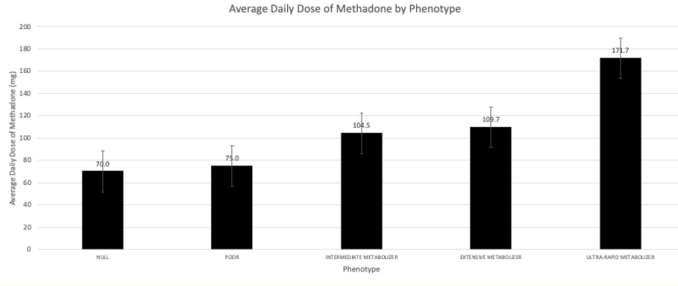

Figure 2: Average daily dose of methadone by phenotype.

Dosage by Ethnicity (Mixed)									
Ethnicity	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**	**Lower Quartile**	**Median**	**Upper Quartile**
Asian	4	4	80.0	9.1	70.0	90.0	72.5	80.0	87.5
Caucasian	9	9	101.9	59.3	37.0	210.0	60.0	90.0	150.0
Mixed	8	8	122.4	57.7	50.0	210.0	80.0	104.5	175.0
Native American/Pacific Islander	2	2	185.0	77.8	130.0	240.0	130.0	185.0	240.0

Table 2: Dosing for major ethnicities.

Conclusion

Pharmacogenomic testing may achieve a better approximation of the daily methadone dose. This testing could help achieve a faster serum steady state and, more importantly, symptom relief. This is a pilot study. This positive correlation between cytochrome P450 polymorphisms and methadone final dosage supports a larger-scale study that may permit effective guidelines in methadone therapy. A guided methadone treatment plan could identify ultra-rapid metabolizer patients to expeditiously get them to therapeutic dose and direct them to additional and alternative therapeutic options. Finding the appropriate maintenance dose can prove to be a complex task, but genotype testing for CYP2D6 could prove to be a helpful tool for physicians in need of quick and objective information. For patients like the "model" patient and patients in other situations, testing can circumvent stigmatization and efforts can be put instead into remedying the patients' other medical and psychosocial issues.

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