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Project Title: Pharmacogenetics Prediction of Metoprolol Effectiveness
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I. Hypotheses and Specific Aims:

We aim to determine if genotyping of the metabolic enzyme CYP2D6 and the drug target ADRB1 will predict response to metoprolol succinate better than the clinical factors that are currently used in clinical practice. Below are the hypotheses and specific aims for this project.

Hypothesis 1: Clinical factors will predict ambulatory systolic blood pressure decrease due to metoprolol better than genotyping of CYP2D6 and ADRB1.
Specific Aim 1: Compare clinical factors (age, sex, race/ethnicity, body mass index, dose, and concurrent medication ingestion) with genotype of CYP2D6 and ADRB1 for prediction of ambulatory systolic blood pressure decrease due to metoprolol 4 weeks after drug initiation.
Hypothesis 2: Metabolomic markers will predict ambulatory systolic blood pressure decrease due to metoprolol better than clinical factors, CYP2D6 genotype, ADRB1 genotype, or CYP2D6 phenotype alone.
Specific Aim 2: Identify urine metabolites associated with ambulatory systolic blood pressure decrease due to metoprolol and determine the relative contribution of the metabolomic markers in a model combining clinical factors, CYP2D6 genotype, and ADRB1 genotype in a stepwise logistic regression model.

II. Background and Significance:

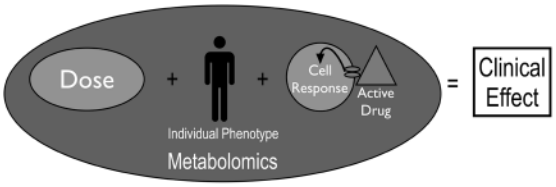
The Importance of Personalized Medicine: 74% of all physician office visits involve drug therapy. These prescriptions accounted for $234.1 billion in patient costs in 2008[5]. 48% of people in the US take at least one prescription and more than 76% of people 60 years or older are on two or more[5-7]. Despite this remarkable utilization of prescription drugs, many of these medications are ineffective. Hypertension (HTN) is the most common chronic medical disease in the US and 64% of patients receiving antihypertensive treatment fail to achieve blood pressure (BP) control[8]. Personalized medicine, defined as the right dose of the right drug for the right patient, has the potential to improve drug efficacy. This can result in cost savings by eliminating time intensive up-titration, eliminating therapies that are destined to be ineffective, and minimizing drug toxicity. Personalized medicine has been difficult to achieve in complex medical conditions due to a variety of factors. Variability in (1) clinical factors including age, sex, race/ethnicity, drug dose, concurrent drug ingestion, and co-morbid disease, (2) genetic variability in drug metabolism, drug targets, and the disease itself, and (3) variability in CYP

metabolism phenotype alters the amount of drug available to the target. It is not surprising that reliance on any single factor has not resulted in widespread achievement of personalized medicine.

Failures of Personalized Medicine: Identification of a single genetic mutation rarely predicts clinical response[9-11]. This is due to the complexity of the interaction between humans, their disease, and the drugs used to treat them[12]. Variability in drug absorption, underlying enzyme capacity, redundancy in enzyme function, transporter polymorphism, receptor variability, drug clearance variability, and medication interactions all contribute to this complex interface[9, 13]. Genomic profiling of hepatic cytochrome (CYP) polymorphisms has been effective at predicting pharmacokinetic relationships in healthy, well-characterized populations with no medication interactions[9, 14-17]. Drug interaction complicates this prediction as demonstrated by Monte *et al*, the presence of CYP2D6 drug interactions predicted the clinical outcome of administered drugs better than the underlying CYP2D6 genotype[18]. This is supported by the observation that area under the curve (AUC) is accurately predicted by CYP genotyping, but it does not accurately predict efficacy[19]. Accounting for several genomic polymorphisms together has improved the prediction of drug response. Genotyping CYP2C9 allowed for physicians to account for 17% of the dosing variability in warfarin therapy. When knowledge of the drug target was added, vitamin K epoxide reductase, 40% of the variability could be accounted for. By adding knowledge of the patient's age and weight, 56% of the variability can be explained[20]. 44% of the dosing variability remains unexplained and this represents the most successful attempt at personalized medicine to date. Failure should have been expected since metabolism phenotype and medication interactions are ignored with this limited genomics approach.

Benefits of an Integrated Approach: The additive approach used in the warfarin example provides a glimpse into how accounting for multiple factors can improve prediction. We must account for the major factors that dictate how much drug is present at the drug target and how the target responds to the drug[21]. See Figure 11.1.3. These major factors can be distilled into three critical points: (1) Clinical factors (2) genetic variability altering metabolism and drug target response drug dose, (3) metabolism phenotype which is affected by alterations in drug absorption, distribution, metabolism and elimination. The simple points, like drug target, can be genotyped and the more complex points, such as absorption, distribution, metabolism and elimination can be phenotyped. Knowledge of all of these contributing factors must be available to realize accurate prediction. In fact, few agents have adequate characterization throughout this interface to allow for this comprehensive integrative approach to personalized medicine.

Figure 11.1.3. Integrative Approach to Personalized Medicine



Metabolomics characterizes complex biologic systems: Metabolomics is an exciting new field that evaluates patterns of small metabolite molecules in biofluid. These metabolites represent the culmination of all upstream processes. Single metabolite associations suffer from the same limitations described for genomic association studies. Many factors contribute to the ultimately observed drug response so prediction of distinct disease patterns, genetic polymorphisms, or environmental factors are bound to be ineffective. Accounting for these processes together is more likely to produce strong associations with drug response[13, 22-25]. To our knowledge, no metabolomic studies have

examined complex diseases and their treatment with precise knowledge of the upstream factors. Characterization of metabolites in the context of a well-characterized biologic system may allow for substitution of numerous upstream tests with a single metabolome assay[26]. **Metabolomic assays may replace clinical, genomic, and/or phenotype factors.**

Metoprolol in an integrated model: Metoprolol is a B1 selective antagonist, known as a beta-blocker. Beta-blockers are first line treatment for heart failure, HTN, angina, and myocardial infarction [27-31]. In 2011, 34.5 million prescriptions for metoprolol were written in the US [32]. Thus, metoprolol is a first line therapy for several of the most common chronic diseases and is one of the most commonly prescribed drugs in the US. There is extensive genomic and pharmacokinetic data available for metoprolol associated with drug response.

Genomic polymorphisms have been associated with metoprolol efficacy. The CYP2D6 is the only clinically pertinent pathway of metoprolol metabolism[33, 34]. CYP2D6 polymorphisms have been associated with altered levels of active metoprolol[1, 34]. Johnson *et al* and Liu *et al* have demonstrated that genetic polymorphisms in the drug target, B1 receptor (ADRB1) have been associated in increased metoprolol efficacy[2, 3]. These polymorphisms do not independently predict efficacy with a high level of efficiency[33]. In addition, medication interactions are known to alter clinical effect and active drug levels[35]. The effect of interactions are stratified by underlying genotype. That is, more drugs are required to inhibit metabolism in patients with high enzyme activity than in patients with low enzyme activity[18]. Intestinal absorption of the drug affects how much drug reaches the liver to be metabolized[36]. A patient's body mass index (BMI) affects distribution and availability to the drug target. The kidney is responsible for only 5% of metoprolol clearance and altered renal elimination has not been shown to alter drug levels[37].

An effective model can be built if clinical factors, genomic and metabolism phenotype data are available in a large cohort of patients. Bioinformatics can be utilized to integrate these associations rapidly and accurately. An integrated approach to predict metoprolol response is possible because genomic and pharmacokinetic studies have been linked with clinical drug response[38]. **Like metoprolol, this integrated approach can predict the clinical response to many drugs as clinical, genomic and metabolism phenotype data become available.**

Application to other treatments: Additional pharmacogenomic and metabolism phenotype association data are published daily. This integrated approach can be applied to any drug when genomic and metabolism phenotype associations linked to clinical outcomes are available. For instance, a model for warfarin dosing could be established if drug target genomics, metabolism phenotypes and the shunting between CYPs can be accounted for utilizing this integrated model. As suggested, future models can mature to account for clinical factors, metabolic shunting, medication interaction, or environmental factors.

Summary of Significance: To achieve personalized medicine, characterization of the key clinical, genomic, and metabolism phenotype factors must be integrated together. The pharmacogenomics and pharmacokinetics of metoprolol have been sufficiently identified to build an integrated model to predict drug efficacy. The framework of this model can be

applied to other therapies as pharmacogenomic and metabolism phenotype information associated with drug response become available.

III. Preliminary Studies/Progress Report:

Monte *et al* have demonstrated that neither dose nor CYP2D6 genotype adequately predict the clinical effect of antiemetics or analgesics that are dependent upon the enzyme in a heterogeneous population[18]. We demonstrated that medication interaction plays a larger role in predicting drug response than genotype alone[18]. We have demonstrated that the drug response was stratified by the presence of CYP2D6 interactions but modified by CYP2D6 genotype in a group of 500 subjects[18, 39]. This alteration by drug interaction is likely due to alteration of enzyme activity due to saturation and inhibition. However, this did not entirely explain the response on an individual patient basis, likely due to variability in clinical factors, the drug target, and metabolism phenotype. This highlights the importance of an integrative approach that accounts for variability at the critical interfaces of drug therapy. We have demonstrated the ability to prospectively enroll a large cohort of patients, obtain demographics, genomic data, and capture longitudinal drug efficacy data. We seek to build a more comprehensive picture of drug response utilizing an integrated approach.

IV. Research Methods

A. Outcome Measures:

Specific Aim 1 Outcome: Metoprolol drug response will be determined by systolic blood pressure (SBP) change from baseline. 10% decrease in SBP will be considered a significant BP change in the individual patients.

Specific Aim 2 Outcome: Again, metoprolol drug response will be determined by SBP change from baseline and a 10% decrease in SBP will be considered a significant BP change. Metabolomic associations with SBP decrease will be determined utilizing partial least squares discriminant analysis followed by orthogonal projections to latent structures discriminant analysis[40]. The most significant five identified factors will be entered into the logistic regression analysis.

B. Description of Population to be Enrolled:

Patients will be referred to the study clinic by either primary care physicians or self-referral from flyers displayed in the clinics and flyers disseminated in other public places. A professional research assistant (PRA) will screen subjects for eligibility via a scripted phone interview and if eligible, a study clinic appointment will be made.

- Inclusion criteria: age >30 years and < 80 years, diagnosis of uncontrolled essential HTN.
- Exclusion criteria: end stage liver disease, end stage renal disease, pregnant females, American Society of Anesthesiologists (ASA) classification of >3, wards of the state, prisoners, decisionally challenged, HR<60 bpm, AV block>240 msec, active reactive airway disease, or illicit drug abuse (excluding marijuana) in the preceding 30 days.

C. Study Design and Research Methods

We will prospectively follow a clinically, genomically, and metabolic phenotypically heterogeneous population of uncontrolled HTN patients beginning metoprolol succinate therapy to determine clinical drug effect. We will be providing the metoprolol succinate study drug to patients at study visits. We will determine each individual's genotype for both CYP2D6 and ADRB1 (Aim 1). Metabolomic markers will be identified to determine if specific metabolites are associated with drug response, genotype variability, or the CYP metabolism phenotype (Aim 2). Finally, the data will be integrated into a stepwise linear regression model to predict drug response. We plan to validate this model in a prospective multi-center study in the future.

Treatment: Subjects will be started on metoprolol succinate, if deemed appropriate by the PI. Resting HR and BP will be determined at study visits. One week after therapy initiation there will be a follow up appointment to ensure no adverse drug events have occurred. Subjects will be asked to avoid coffee or other stimulants 4 hours prior to the study visit. Up-titration of the medication will be performed at this follow up visit as needed to meet the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of HTN[27] (JNCVIII) BP goal of <140/90. Titration will be performed utilizing the algorithm identified in Table 1. At the initial study visit, patients will be consented. Eligibility will be confirmed. Metoprolol will be started if deemed appropriate at initial visit, either in the ED or in the study clinic. Patients will have their blood pressure taken, medical history taken and confirmed with medical record review, blood taken for genetic testing, and urine taken for metabolomic analysis. A 2 week supply of

Table 1: Metoprolol titration during treatment phase

ABP, adjusted if either SBP or DBP out of range	Metoprolol succinate dose
≥140/90	50 mg once daily
≥160/90	100 mg once daily
≥180/90	150 mg once daily
≥200/90	200 mg once daily

Table 2: CYP metabolizer status and associated pharmacokinetic changes[1].

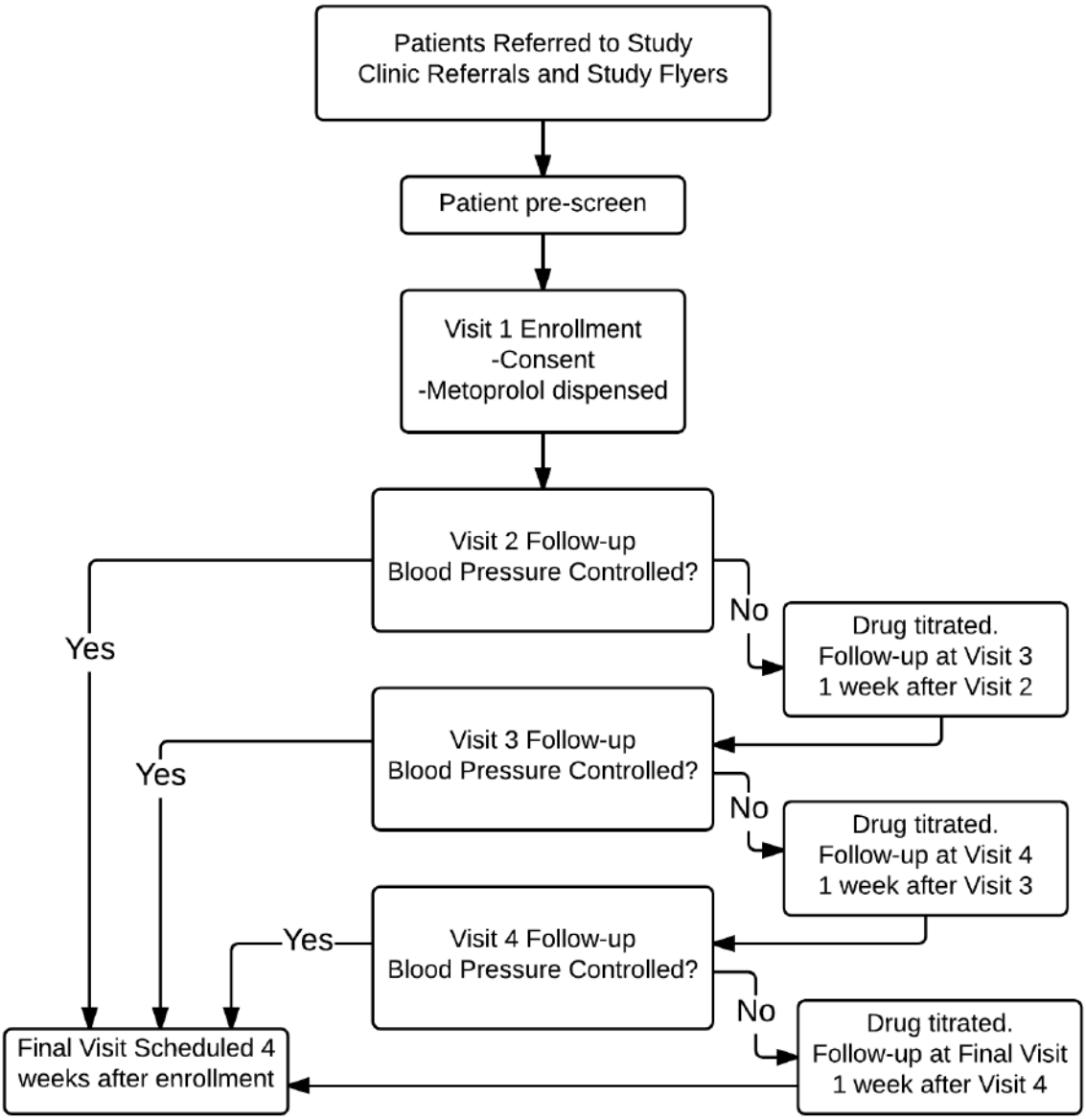
CYP2D6 metabolizer status	Change in clearance	Activity Score[4]
Poor metabolizer (PM)	5 fold decrease	<1
Intermediate/Extensive metabolizer (IM/EM)	0.8-1.0 (EM is wild type)	1-2
Ultra rapid metabolizer (UM)	2 fold increase	>2

metoprolol will be dispensed. We will schedule a follow up at the 1 week mark but will dispense 2 weeks of study drug to allow for the subject to reschedule if necessary. At the 1 week follow up (visit 2), we will ensure treatment is compliant with JNC-VIII hypertension guidelines. If not, they will be prescribed the appropriate therapy and asked to return in 1 week for a blood pressure re-check (week 2 follow up or visit 3). Only one blood pressure medication change will occur at each study visit. At each follow up visit, blood pressure will be taken and urine will be obtained for repeat metabolomic analysis (see below). If the subject runs out of study drug prior to a visit (due to missed appointments or loss of the drug, for example), the subject will be given a 1 week supply of study drug and the study visit will be rescheduled in one week. No samples will be collected if the patient has not been taking the study drug. The rescheduled visit will be considered a continuation of the prior visit since no medications will be changed and no samples will be obtained. Patients must have taken 5 doses of the drug to ensure steady state at the follow up visit.

Blood pressure control will be considered <140/90. See Figure 1 for patient flow through the titration phase. If subject's blood pressure is controlled at Visit 2, subject will be given a 3 week supply of metoprolol and scheduled for final appointment 2 weeks from Visit 2. Note: Subject is given 3 week supply to allow for scheduling problems. (Figure 1). If blood pressure is not controlled, patient will have metoprolol titrated up (see titration Table 1) and will return for follow up at week 2. HR < 60 bpm will preclude further titration regardless of BP. Titration will be continued until either the blood pressure is controlled or **week 4** of metoprolol is reached. See Figure 1. We will continue to titrate the other anti-hypertensives for an additional 2 weeks and those that have not had their blood pressure controlled by metoprolol titration after 6 weeks will be referred back to their PCP. Urine and blood pressure will be gathered at each follow-up visit. **At week 4**, 2 additional months of metoprolol, the dose necessary to control the blood pressure, will be dispensed. Compliance will be monitored by patient drug ingestion history urine metabolite detection (See Aim 2: Metabolomic Analysis).

Clinical Factors: Clinical factors known to be associated with altered metoprolol response will be captured. Male gender, African American race, higher BMI[41], lack of CYP2D6 co-ingestants[42], and lower dose[43, 44] has been associated with uncontrolled HTN. Shelley *et al* demonstrated the effect of diabetes was eliminated when the non-diabetic blood pressure of 140/90 mmHg was considered controlled rather than the lower recommended blood pressure for diabetic patients[41]. Diabetes will not be included as a co-variate to maintain a standard definition of BP control.

Figure 1. Patient flow through Protocol.



Genotyping: CYP2D6 and ADRB1 variants will be identified using long range PCR (XL-PCR) and PCR restriction fragment length polymorphism (PCR-RFLP). This technique accounts for allele variants and multiplication and is inexpensive, easy, efficient, and reliable in patients independent of ethnicity or race[4]. Twenty-six CYP2D6 variants will be identified and activity scores will be assigned based upon variant identification as described by Gaedigk *et al* (see Table 2)[4]. We will identify the 4 ADRB1 variants outlined in Table 3. Identifying these extreme discordant phenotypes accounts for clinically significant polymorphisms[45, 46].

Table 3: ARB1 variants and associated metoprolol clinical effect.

ARB1 genotype	Metoprolol clinical effect
49Ser389Arg/49Ser389Arg	3 fold greater DBP reduction[2, 3]
49Ser389Arg/49Gly389Arg	Good responder[3]
49Ser389Gly/49Gly389Arg and 49Ser389Gly/49Ser389Gly	Non-responders[2, 3]

Metabolomic analysis: Metabolomics is the unbiased global survey of all the low molecular-weight molecules or metabolites in a biofluid. These metabolites are the final downstream products of genomic, transcriptomic, and/or proteomic perturbations[25]. In clinical settings, urine is the ideal biofluid for analysis since it is non-invasive to obtain, accounts for alterations in renal clearance, and it does not require removal of cellular debris. Urine samples will be utilized for metabolite detection. The metabolite detection will be performed utilizing ultra high performance liquid chromatography electron ion spray and qualitative time of flight mass spectrometry.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Coercion is a potential risk given that subjects will be given 3 months of study drug and monetary compensation for the pharmacokinetic portion of the study. The monetary value of the drugs and re-imbursement are small. This re-imbursement strategy has been used by others at UCD[16, 47-49] with success in enrollment and this has been deemed an appropriate monetary reimbursement by our IRB.

Adverse drug events (ADEs) to metoprolol are possible in this open label clinical trial design.

Classification of Risks Rare, serious:

- Congestive heart failure, heart block, and bronchospasm are serious ADEs associated with metoprolol succinate therapy. Exclusion criteria utilizing the ASA classification have been developed to specifically guard against these risks. Subjects that have severe co-morbid disease that is a constant risk to life will be excluded, determined ASA classification of >3.

Common, minor:

- Venipuncture is associated with some temporary discomfort and possibly bruising. This minor procedure is not known to be associated with risk of substantive harm.
- Bradycardia or hypotension are inherent risks of metoprolol therapy. Titration of the drug will be performed weekly to minimize the risk of these ADEs.
- Hypersensitivity reactions to either metoprolol is possible. Most of these reactions are mild rashes, itching, abdominal pain, or nausea.

The study safety officer (Dr. Heard) independent from the research team will review the clinical effects documented every 6 months and within 48 hours for any serious ADE.

Breach of confidentiality is a risk to subjects though significant effort will be made to mitigate this risk. Genomic studies contain their own inherent risk in the identification of potentially clinically important polymorphisms. Both the subject and their medical provider, if desired by the subject, will be informed of the subject's CYP metabolizer status. Information will be given by written communication and direct phone contact.

Adequacy of Protection Against Risks. Subjects will be referred to the study through fliers in clinics and suggestion by primary care physicians. Primary care physicians will not be involved in the collection of data or the review of patient charts for the study and thus will not be in a position to coerce subjects. To further minimize coercion, a Research Subject Advocate (RSA) will be present to observe the consent procedure for a subset of subjects enrolled and give study staff feedback. There will be no exchange of payment for study participant referrals, and we will not offer payment or other compensation to primary care physicians. Subjects will be in a supervised medical setting and monitored by study staff during study medication dispensing visits and interviews. Study staff will also be notified of any participant-initiated complaints or potential adverse events.

The consent will be reviewed in detail by the PI or PRA then subjects will be given as much time as they desire to independently review the form during which time the PI and PRA will review the consent form with other potential subjects. When the subject is comfortable, they've had a chance to ask questions, and they are able to recapitulate the major purpose of the study and the potential risks/benefits if they participate then they will be asked to sign the form in the presence of the PI.

Breach of confidentiality will be mitigated utilizing the REDCap HIPAA compliant database for data collection. Hard copies of consent will be stored in a locked filing cabinet in the PI's office on a card-accessed floor.

Patients starting metoprolol therapy have the inherent risks of adverse drug events (ADEs). These risks will be mitigated by strict adherence to the exclusion criteria and follow up visits with a specific questionnaire to screen for ADEs (see data collection form XX). UCH medical records will be reviewed and all visits, clinic or ED, will be reviewed to ensure not ADE to the metoprolol study drug 3 months after initial enrollment. All ADEs will be captured by the professional research assistants and reviewed by the PI and the safety officer, Dr. Heard.

E. Potential Scientific Problems:

Enrollment of 500 subjects with uncontrolled HTN may be difficult from primary care clinics. We have attempted to mitigate this potential problem by using the ED and multiple clinics to draw patients from. The UCH adult clinics saw 3,588 uncontrolled hypertensive patients in 2012 from which we will draw. The MCPN clinics saw over 34,400 medical patients in 2011. MCPN has received a 1.2 million dollar grant under the Affordable Care Act to increase access to care in Aurora, CO where UCH is located. This grant is expected to increase new patient visits to MCPN and increase their access to the

University health system[50]. Enrollment of 500 subjects represents enrollment of 3.3 patients per week for the initial 3 years of the study.

F. Data Analysis Plan:

Metoprolol drug response will be determined by systolic blood pressure (SBP) change from baseline. 10% decrease in SBP will be considered a significant BP change in the individual patients. This change is consistent with prior CYP metabolizer and ADRB1 variant status studies[2, 34]. In addition to the clinical demographic variables potentially affecting metoprolol response (smoking status, co-morbid disease, and the presence of other antihypertensive therapy) will be captured and assessed by logistic regression analysis. A receiver operator characteristic (ROC) will be calculated for the model. A second logistic regression will be generated using genotype as predictor variables. Expected CYP2D6 metabolizer status will be determined with the CYP2D6 activity score as outlined in Table 2[4]. Activity scores will be entered into the model as a continuous variable. ADRB1 genotype (See Table 3) will be included as an ordinal variable. Again, a ROC will be calculated and the ROCs will be compared utilizing area under the curve (AUC) to determine which factors, clinical or genotypes, are most efficient to predict SBP decrease due to metoprolol. We will ensure that each of the clinical and genotype factors contribute to the respective models by calculating likelihood ratios for each variable. This will ensure there is no co-linearity in the factors entering the models. We do not expect co-linearity between these factors based upon prior investigators work, however, if there is co-linearity, redundant factors will be eliminated from the model. Based upon an expected ROC AUC of 0.7 for clinical factors[41-44] and 0.6 for genotyping[1, 2] the power calculation determines that 212 are needed patients to have 80% power at the 0.05 alpha level. Given that the prevalence of the CYP2D6 genotype is possibly as low as 3%, we will enroll a total of 500 patients to ensure adequate power for Aims 2 as well.

Metabolomic associations with SBP decrease will be determined utilizing partial least squares discriminant analysis followed by orthogonal projections to latent structures discriminant analysis[40]. The most significant five identified factors will be entered into the logistic regression analysis. We will utilize a stepwise logistic regression model including clinical factors, CYP2D6 genotype, ADRB1 genotype, and metabolomic markers. It is likely that there will be co-linearity between the metabolomic and explanatory variables. Therefore likelihood ratios will be calculated for each variable entering the model and only factors adding additional explanation will be included. Both forward and backward stepwise regression will be explored to find the best model fit. This integrative approach is likely to demonstrate an extremely high ROC to predict SBP decrease due to metoprolol.

G. Summarize Knowledge to be Gained:

Potential Benefits of the Proposed Research to Human Subjects and Others.
Subjects will benefit from knowing their CYP2D6 and ADRB1 genotypes since drug efficacy has been associated with polymorphism in these genes. Many drugs are dependent upon CYP2D6 metabolism thus many drug therapies may be altered with this information in hand. Primary care physicians will be given this information to facilitate more effective drug therapy for their patients. A model that predicts metoprolol efficacy can eliminate ineffective treatment and minimize time consuming and costly up-titration of the drug for patients.

This is the first study of its kind integrating the multiple contributory factors to drug efficacy. This approach views the individual patient response as a sum of the parts thereby improving the safety and efficacy of drug therapy. This information can limit drug-drug interactions for these patients in the future.

The potential benefits to society are high. This model can be applied to other drug therapies as the pharmacogenomic data becomes available. Models that predict drug efficacy and safety prior to prescription save health care visits, eliminates the risks of ADEs, and minimizes the need or up-titration. Sooner effective drug therapy can yield immeasurable cost savings by preventing complications and minimizing additional office visits and hospital admissions.

Importance of the Knowledge to be Gained. Metoprolol is one of the most commonly prescribed drugs in the United States and is first line therapy for hypertension. Ineffective therapy with this drug places patients at risk for ADEs and is costly. These risks and costs can be eliminated if the drug is destined to be ineffective. A model that integrates clinical, genomic, pharmacokinetic, and metabolomic data is more likely to predict drug efficacy and safety with a high degree of success than any single factor alone. Most importantly, this integrative model can be applied to other therapies in order to mitigate these same risks and costs. Other drugs are far more costly and some are exceedingly toxic in the wrong patient. When serious ADEs occur significant costs are incurred. A model that identifies patients at risk of ineffective drug therapy or those at risk of serious ADEs prior to therapy can represent incredibly important knowledge for physicians and drug approval authorities.

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