GENETIC VARIATION OF THE BETA-2 ADRENERGIC RECEPTOR AND THE
PHENYLETHANOLAMINE N-METHYLTRANSFERASE ENZYME: INFLUENCE
ON CATECHOLAMINES, CARDIOVASCULAR REGULATION, AND THE
CARDIOPULMONARY RESPONSE TO ALBUTEROL

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Marina G. Martinez

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Marina G. Martinez, titled Genetic Variation of the Beta-2 Adrenergic Receptor and the Phenylethanolamine N-Methyltransferase Enzyme: Influence on Catecholamines, Cardiovascular Regulation, and the Cardiopulmonary Response to Albuterol and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

________________________________________  Date: January 21, 2014
Eric Snyder, PhD

________________________________________  Date: January 21, 2014
John Konhilas, Ph.D.

________________________________________  Date: January 21, 2014
Zoe Cohen, Ph.D.

________________________________________  Date: January 21, 2014
John Regan, Ph.D.

________________________________________  Date: January 21, 2014
David Nix, Pharm.D.

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

________________________________________  Date: January 21, 2014
Dissertation Director: Eric Snyder, Ph.D.

________________________________________  Date: January 21, 2014
Dissertation Director: John Konhilas, Ph.D.
STATEMENT BY AUTHOR

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SIGNED: Marina G Martinez
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DEDICATION

This dissertation is dedicated to the memory of my parents, Marina and Jose Luz Martinez.
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ABSTRACT

Hypertension, or chronic blood pressure elevation, affects approximately a third of American adults and is responsible for $70 billion dollars annually in medical costs. Recent studies have attempted to identify genetic variants that influence cardiopulmonary function, including blood pressure regulation. This study seeks to determine whether a polymorphism in position -182 of the gene encoding the phenylethanolamine N-methyltransferase (PNMT) enzyme, which converts norepinephrine to epinephrine, influences catecholamine levels and cardiovascular function. Secondly, this study seeks to explore whether a polymorphism at amino acid position 16 of the beta-2 adrenergic receptor (B2AR) affects the cardiovascular response to albuterol in healthy individuals; this study also explores the pulmonary response to albuterol in healthy subjects and patients with cystic fibrosis according to B2AR genotype. All subjects were genotyped and stratified according to genotype. Baseline measurements were taken. Albuterol was administered via nebulizer. Cardiopulmonary measurements were taken again at 30-, 60-, and 90- minutes post-albuterol administration. This study found that the PNMT polymorphism at position -182 influences circulating epinephrine, the epinephrine:norepinephrine ratio, and cardiac output. The B2AR polymorphism at amino acid position 16 affects the percent change in systemic vascular resistance in response to albuterol administration in healthy subjects. Furthermore, this study found that the B2AR polymorphism at amino acid 16 affects the change in forced vital capacity following albuterol administration in cystic fibrosis subjects.
CHAPTER 1: INTRODUCTION

1.1 Hypertension and the regulation of blood pressure

Mean arterial blood pressure is the main driving force for adequate tissue perfusion. Blood pressure must be regulated for two main reasons\(^1,2\). First, blood pressure must be high enough to ensure appropriate blood flow to tissues and second, blood pressure must be maintained at a level that will prevent vascular damage and overexertion of the heart\(^3\). The regulation of blood pressure is a complex, multifactorial process involving coordination of the cardiovascular system, the renal system (through the control of blood volume) and the sympathetic nervous system\(^4,5\). The two major determinants of mean arterial pressure, defined as the average blood pressure in an individual, are cardiac output and total peripheral resistance\(^3\). Cardiac output itself depends on stroke volume and heart rate. Heart rate lies mainly under the control of direct innervation by the autonomous nervous system and also circulating catecholamines. Stroke volume, like heart rate, is also influenced by the parasympathetic and sympathetic nervous systems and catecholamines. However, stroke volume is also determined by venous return, a factor altered by salt and water balance as dictated by the renal system. The second major determinant of mean arterial pressure is total peripheral resistance (TPR). Blood viscosity, a reflection of red blood cell number, is the one determinant of TPR. However, arteriolar radius is the most important factor driving TPR; overall, viscosity does not change. Two main determinants of arteriolar radius exist. The first is local control via metabolites released by skeletal muscle seen during
exercise. The second is extrinsic control, mainly though the vasoconstricting effects of the sympathetic nervous system and the renin-angiotensin-aldosterone system. The determinants of blood pressure are illustrated in Figure 1 below, as adapted from Sherwood’s Human Physiology textbook\(^3\).

![Diagram of blood pressure determinants](image)

**Figure 1:** Determinants of mean arterial blood pressure (ANS: Autonomic Nervous System, RAAS: Renin Aldosterone Angiotensin System).

Two mechanisms help maintain blood pressure at adequate life-sustaining levels: short-term regulation through the baroreceptor reflex and long-term regulation through adjustment of blood volume via salt-water balance. In hypertension, a condition of sustained blood pressure elevation, these mechanisms become dysfunctional and cannot successfully maintain blood pressure at adequate levels. Blood pressure is considered normal if under
120/80 mmHg. Figure 2 illustrates the stages of hypertension; stage 1 hypertension is defined as systolic blood pressure readings of 140-159 mmHg and diastolic blood pressure of 90-99 mmHg. Systolic blood pressure is the pressure in blood vessels during cardiac contraction whereas diastolic blood pressure is the blood pressure during cardiac relaxation.

<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic</th>
<th>Diastolic</th>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120-139</td>
<td>80-89</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>≥160</td>
<td>≥100</td>
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**Figure 2:** Categories for blood pressure levels in adults (measured in millimeters of mercury, or mmHg). Figure adapted from the National Institutes of Health (2013).

This present study focuses on the phenylethanolamine N-methyltransferase (PNMT) enzyme, converter of norepinephrine into epinephrine, and the beta-2 adrenergic receptor (B2AR). As alluded to above and discussed further in the following sections, interactions between epinephrine/norepinephrine with the B2AR cause physiological alterations that affect mean arterial blood pressure and can ultimately lead to hypertension.
1.2 Societal burden of hypertension

Hypertension, a condition of chronic blood pressure elevation, affects 68 million people in the United States, approximately one third of American adults (National Center for Health Statistics 2011). Known as the “silent killer”, this condition is responsible for $70 billion dollars annually in direct medical costs in the United States. Over 1 billion people worldwide have hypertension and the World Health Organization predicts this number will reach 1.5 billion by the year 2025 due to increases in both population size and number of people reaching older ages. These numbers are especially alarming considering the medical complications that arise from prolonged elevations of blood pressure.

Hypertension is a major risk factor for heart disease and stroke, the first and third leading causes of death in the United States, among other life-threatening conditions such as renal disease. Previous studies have shown direct correlations between levels of systolic and diastolic pressure with risk of coronary heart disease and stroke. Hypertension also poses a danger to pregnant women as it is the most common medical complication encountered during pregnancy. Furthermore, hypertensive disorders are the second most common cause of maternal death in developed countries. Overall, blood pressure related disease kills 8 million people every year.

1.3 Monogenic vs. Essential Hypertension

Like most complex genetic diseases, the onset of hypertension is most likely dictated by the interaction between genes and the environment.
Environmental factors include age, gender, diet, physical activity and race. These factors are equally important as genetic factors, are often difficult to quantify, and ultimately are outside the scope of this study. Investigation of genetic basis of high blood pressure began with the work of George Pickering and Robert Platt in the 1940s. Platt’s work ultimately described monogenic forms of hypertension in which rare genetic mutations have a drastic effect on blood pressure. Liddle’s syndrome, for example, results in blood pressure increases due to a gain of function mutation in the gene encoding the epithelial sodium channel\textsuperscript{14}. In this case, hypertension is a result of elevation in sodium and fluid reabsorption through the distal tubule of the nephron, which leads to an increase in blood volume and, hence, blood pressure\textsuperscript{15}. In contrast to Platt’s work, Pickering described the collective effect of multiple gene variants, each with a small yet significant effect on overall blood pressure. Overall, Pickering’s work described the polygenic nature of essential hypertension that makes up the majority of hypertension cases\textsuperscript{16}.

1.4 Genetics of blood pressure regulation: Genetic approaches to identifying genetic variants that influence the regulation of blood pressure

The development of genetic tools has helped expand Pickering’s idea of genetic contributors to essential hypertension. Three approaches dominate the research of blood pressure genetics: Candidate gene studies, genome wide linkage scans, and genome wide association studies.
Candidate gene studies investigate gene variants based on prior biological information. Genes are selected for candidate studies based on their known physiological role on blood pressure regulation; therefore, any genes and their containing variants that do not possess an obvious link to blood pressure regulation are ignored. Renal sodium handling genes (such as the epithelial sodium channel) and genes within the renin-angiotensin-aldosterone system have been identified as candidate genes of essential hypertension\(^\text{17-21}\). Additionally, genes involved in vasodilation and vasoconstriction, including nitric oxide synthase and endothelin genes, have been associated with long-term blood pressure regulation\(^\text{22-24}\).

A second genetic approach is genome-wide linkage scans; this method in part addresses the drawback of candidate gene studies of needing a priori knowledge. As the name suggests, this approach is based on the concept of genetic linkage, the tendency for genes within the same chromosome to be inherited together. Genome-wide linkage scans have found quantitative trait loci of blood pressure on every chromosome. Quantitative trait loci are regions within a chromosome that contain a gene or genes that may influence quantitative traits such as blood pressure\(^\text{25}\). Genes identified by genome-wide linkage scans include those encoding the angiotensin converting enzyme and \(\beta\)-adducin\(^\text{25-28}\). However, findings identified by this genetic approach generally have not been replicated.

Genome-wide association studies interrogate common polymorphisms, defined as those with a minor allele frequency of at least 5%, for association with
blood pressure and hypertension. Because multiple polymorphisms can be tested with SNP chips (millions per test), genome wide association studies are subject to a multiple-testing burden; this results in a very conservative significance level of $\alpha < 5 \times 10^{-8}$\textsuperscript{29}. These studies cannot be replicated between differing populations (i.e. European vs. non-European descent) due to differences in allele frequencies and patterns of linkage disequilibrium\textsuperscript{30}. Another characteristic of this test is the requirement of a large sample size, a limitation overcome by the use of meta-analysis of multiple genome-wide association studies. This genetic approach has uncovered multiple candidate genes for hypertension, including some less obvious genes with no straightforward role in blood pressure regulation. Some of these genes include the uromodulin gene (uromodulin is found in the kidney and has a role in fluid reabsorption), genes coding for calcium-gated channels, and the gene for the beta-1 adrenergic receptor\textsuperscript{31-33}.

The genes of interest in this study have been identified as relevant to blood pressure regulation and with common variants in the population. The specific location of the PNMT gene in human chromosome 17 (murine chromosome 10) is found within a gene region that has been associated with blood pressure regulation\textsuperscript{34, 35}. Similarly, the gene for the beta-2 adrenergic receptor has been linked to the regulation of blood pressure\textsuperscript{36-38}. 

1.5 Phenylethanolamine N-methyltransferase enzyme and its role in catecholamine formation

The phenylethanolamine N-methyltransferase (PNMT) enzyme plays a principal role in the formation of catecholamines which act as sympathetic agents in our bodies. This enzyme catalyzes the formation of epinephrine by directing the transfer of a methyl group from S-adenosyl methionine to the amino group of norepinephrine (Figure 3)\textsuperscript{39, 40}. PNMT can be found in various regions of the body, including the retina, brain, and heart; however, PNMT in the adrenal medulla is the principal site for the formation of epinephrine, a principal sympathetic regulator of our organ systems\textsuperscript{41, 42}.

\textbf{Figure 3:} Conversion of norepinephrine into epinephrine by the PNMT enzyme.

The adrenal medulla is considered a modified sympathetic ganglion; similarly, the chromaffin cells it contains are considered modified post-ganglionic
sympathetic neurons. During a sympathetic activity such as exercise, sympathetic efferent fibers in contact with the adrenal medulla release acetylcholine. Acetylcholine triggers the release of catecholamines from chromaffin cells within the adrenal medulla (Figure 4)\textsuperscript{43}. Epinephrine makes up the majority of medullary output during sympathetic stimulation. Approximately 80 percent of medullary output consists of epinephrine while the rest is composed of norepinephrine\textsuperscript{44}. While the adrenal medulla is the sole source of circulating epinephrine, the majority of norepinephrine in the bloodstream comes from spillover from sympathetic nerve fibers\textsuperscript{45}.

\textbf{Figure 4: Sympathetic stimulation of the adrenal medulla.}
1.6 Physiological effects of catecholamines, the products of PNMT

Epinephrine and norepinephrine, two of the three systemic catecholamines, have multiple functions in the human body and collectively make up the fight-or-flight response of the sympathetic nervous system. These two catecholamines cause cardiovascular alterations through their binding to adrenergic receptors in the heart and systemic vasculature. Epinephrine and norepinephrine can bind to beta-1 adrenergic receptors to increase heart rate. Binding of catecholamines to both beta-1 and beta-2 adrenergic receptors increase cardiac contractility. Together, increases in chronotropy (heart rate) and inotropy (contraction) increase cardiac output. In the vasculature, catecholamines bind to alpha- and beta-adrenergic receptors to mediate vasoconstriction and vasodilation, respectively. In the kidney, catecholamines bind to alpha-adrenergic receptors in the proximal tubule cells. This causes upregulation of both the apical sodium-hydrogen exchanger 3 and the basolateral sodium-potassium pump; this results in an overall increase in sodium reabsorption independent of any other hemodynamic effects. Catecholamines also increase the resistance of afferent and efferent arterioles in the nephron, decreasing renal blood flow and glomerular filtration rate (GFR). When the nephron encounters alterations in GFR and therefore changes in sodium flow, the proximal tubule responds by reabsorbing a proportional fraction of the sodium load, a process called glomerular-tubular (GT) balance. Because catecholamines decrease GFR and the filtered load of sodium, sympathetic tone decreases the absolute reabsorption of sodium. Sympathetic stimulation of the kidney also causes renin
release from the granular cells of the juxtaglomerular apparatus. Renin, part of the renin-angiotensin-aldosterone system, leads to elevated levels of angiotensin II, the most potent vasoconstrictor of small arterioles, with the overall effect being elevations in blood pressure.\textsuperscript{47}

The interaction of catecholamines on the cardiovascular and renal systems can ultimately affect blood pressure through their effects on cardiac output, vasoregulation, and sodium/fluid balance. Catecholamines can also, however, affect the pulmonary system; their interaction is relevant to one portion of this study in which the effect of a beta-agonist on pulmonary function is investigated. In the lungs, catecholamines can bind to beta-2 adrenergic receptors to increase bronchodilation and lung fluid clearance.

1.7 Genetic variation of PNMT

This study focuses on a commonly studied polymorphism of the PNMT enzyme found in position -182 (rs\#876493) of the gene promoter region (Figure 5). Common polymorphisms are defined as those with minor allele frequencies greater than 5% in the general population\textsuperscript{48,49}. The polymorphism consists of an adenine (A) to guanine (G) substitution and has a minor allele frequency of 30% in whites and 70% in blacks\textsuperscript{50}. This results in three possible genotypes for this polymorphism: AA, AG, and GG. Multiple studies support the relevance of this polymorphism to physiological function. The A allele at PNMT position -182 has been associated with lower urine epinephrine and decreased in-hospital mortality from acute kidney injury\textsuperscript{51}. Furthermore, position -182 of PNMT has been shown
to influence blood pressure in African Americans, with the G allele tending to be more prevalent in hypertensives\textsuperscript{52}. Most recently, the GG genotype at PNMT position -182 was associated with higher resting venous norepinephrine; the same study also associated this polymorphism to a 39% smaller increase in venous norepinephrine during exercise\textsuperscript{50}.

*Figure 5:* Location of PNMT polymorphism (rs876493) in the gene promoter region (Arrow points to polymorphism at position -182, cited here as position -161; however, rs number verifies -161 and -182 are identical).

These results collectively suggest that genetic variation of the PNMT enzyme at promoter position -182 could have an effect on blood pressure regulation. In fact, two animal models of hypertension, the Doca salt-sensitive rat and the spontaneously hypertensive rat, show elevations of PNMT activity\textsuperscript{53-55}. Furthermore, blood pressure decreases in these animals when given a PNMT inhibitor drug\textsuperscript{53}. Pheochromocytoma, a neuroendocrine tumor of the adrenal
medulla, is a condition characterized by elevations in circulating epinephrine that is accompanied by hypertension\textsuperscript{56, 57}. These studies reinforce the link between PNMT, epinephrine, and blood pressure regulation.

1.8 Role of the beta-2 adrenergic receptor in sympathetic mediation of the cardiovascular system

The beta-2 adrenergic receptors (B2ARs) are important sympathetic mediators of the cardiovascular system. B2ARs mediate cardiac contractility in conjunction with the beta-1 adrenergic receptors; the beta-1 receptor: beta 2 receptor ratio is 70:30 in the atria and 80:20 in the ventricles\textsuperscript{58}. The B2ARs are also present in the vasculature where they help mediate vasodilatory responses to circulating catecholamines.

The B2AR is a classical G-protein coupled receptor with 7 transmembrane domains complete with an extracellular amino domain and a cytoplasmic carboxyl terminus\textsuperscript{59}. Sympathetic agents epinephrine and norepinephrine are both agonists for the receptor; however, epinephrine has an affinity for the receptor 10-50 times greater compared to norepinephrine\textsuperscript{46}.

Agonist stimulation of the B2AR by epinephrine or norepinephrine triggers a signaling cascade leading to cardiac contraction. When an agonist binds to the B2AR, adenylyl cyclase is activated; active adenylyl cyclase converts ATP into cAMP. This allows cAMP to activate phosphokinase A (PKA) by binding to its regulatory subunits; the direct result is the activation of PKA’s catalytic subunits. Active PKA phosphorylates several key proteins that alter calcium cycling to
ultimately increase contractility: L-type calcium channels, ryanodine receptors, and the sarco/endoplasmic reticulum calcium ATPase. PKA phosphorylates L-type calcium channels found in t-tubules thus increasing their open probability. The resulting increase in calcium flux into cardiomyocytes triggers more calcium flux from the sarcoplasmic reticulum (SR) into the cytosol, a process known as calcium-induced calcium release (CICR). CICR occurs when calcium entering cardiomyocytes from L-type calcium channels stimulates ryanodine receptors in the membrane of the sarcoplasmic reticulum, resulting in calcium release from within the SR. Furthermore, active PKA formed from B2AR signaling can also phosphorylate ryanodine receptors to increase calcium flux from inside the SR to the cytosol. Active PKA can also phosphorylate phospholambam. Phospholambam, in its un-phosphorylated state, acts as an inhibitor of the sarco/endoplasmic reticulum calcium ATPase (SERCA). The role of SERCA is to re-sequester calcium back into the sarcoplasmic reticulum from the cytosol during cardiac relaxation. Therefore, when phospholambam is phosphorylated, its inhibitory effect on SERCA is released; the direct result is SERCA’s increased sequestration of cytosolic calcium which translates to increased calcium release into the cytoplasm to mediate enhanced myocardial contraction.

Agonist stimulation of the B2AR also causes relaxation of the systemic vasculature. The increase in cAMP from agonist binding inhibits myosin light chain kinase (MLCK). Normally, MLCK phosphorylates myosin light chains to promote cross-bridge formation between myosin and actin and thus
vasoconstriction. Therefore, B2AR-mediated inhibition of MLCK decreases contractile force and promotes vasodilation.

Overall, stimulation of B2ARs by catecholamines can lead to increases in cardiac output and vasodilation, two principal determinants of blood pressure regulation.

1.9 Role of the beta-2 adrenergic receptor in sympathetic mediation of the pulmonary system

B2ARs are also present in the pulmonary system, with receptor numbers increasing with every airway generation. In fact, 90% of B2ARs in the lung are found in the alveoli. In alveoli, B2AR stimulation enhances lung fluid clearance through PKA phosphorylation of three proteins: the epithelial Na channel (ENaC), the cystic fibrosis transmembrane conductance regulator (CFTR) and the Na-K ATPase. Upregulation of these three key proteins causes fluid to exit the lung, thus clearing excess fluid from alveolar airspace. For this reason, B2AR agonists such as albuterol are used for pulmonary edema of multiple etiologies.

B2ARs in pulmonary smooth muscle behave similarly to receptors in the systemic vasculature; when agonist stimulated, formation of cAMP inhibits MLCK promoting relaxation of pulmonary smooth muscle.
1.10 Genetic variation of the beta-2 adrenergic receptor

This study focuses on a B2AR polymorphism in nucleotide 46 that results in an amino acid substitution at position 16 of the receptor’s extracellular region (Figure 6). The polymorphism leads to an amino acid substitution from arginine (Arg) to glycine (Gly). Three genotypes exist for this polymorphism: AA, AG, and GG. Similar to the PNMT polymorphism, previous studies have shown that the B2AR polymorphism at amino acid position 16 has functional consequences. Subjects with a GG genotype display higher stroke volume and cardiac output both at rest and during exercise\textsuperscript{70}. In addition, the glycine homozygous condition has been associated with sustained vasodilation and decreased desensitization in response to beta-agonist administration\textsuperscript{71, 72}. Despite the increased vasodilation associated with glycine at amino acid position 16 of the B2AR, this polymorphism has been implicated with hypertension\textsuperscript{73}. This can be explained by increased sodium (and thus fluid) reabsorption attributed to glycine at position 16 (B2ARs reside in the kidney collecting duct)\textsuperscript{37}. From a pulmonary standpoint, the glycine homozygous condition is attributed to decreased lung fluid accumulation after administration of a saline load\textsuperscript{74}. 
Figure 6: Location of polymorphisms of the beta-2 adrenergic receptor. Arrow points to polymorphism at amino acid position 1675.

1.11 Genetic variants and therapeutics: Albuterol and genetic variation of the B2AR

A final focus of this study is the interaction of genetic variation of the beta-2 adrenergic receptor with the pharmacological response to albuterol. Inhaled bronchodilators such as albuterol are common treatments for respiratory conditions including asthma and cystic fibrosis. Though use of albuterol is commonplace in clinical settings, its effectiveness is variable among patients. However, albuterol has been shown to improve forced expiratory volume in one second (FEV$_1$); FEV$_1$, a marker for disease status and prognosis in CF, is defined as the amount of air that can be forcibly blown out in one second following a maximal inhalation76-78. To this date, there has yet to be a study on
the effect of genetic variation of the beta-2 adrenergic receptor on the cardiopulmonary response to albuterol.

1.12 Present study

First, this study explores the effect of PNMT genetic variation at position -182 of the gene promoter region. Specifically, this study explores whether the polymorphism has an effect on blood pressure regulation; in order to do this, this study explores the effect of the PNMT polymorphism on circulating catecholamines, cardiovascular function, and renal function.

Secondly, this study explores the effect of genetic variation of the B2AR. Specifically, this study seeks to determine whether the polymorphism has an effect on the cardiovascular response to albuterol. A previous study in our laboratory showed an increase in circulating catecholamines with the administration of nebulized albuterol, accompanied by a decrease in systemic vascular resistance and increases in heart rate and cardiac output; this suggests that genetic variation of the B2AR could influence whether or not albuterol leads to cardiovascular alterations, and to what extent. Furthermore, this study seeks to determine the effect of genetic variation of the B2AR on the pulmonary response to albuterol. Because albuterol is administered to improve pulmonary function in respiratory disease, this study seeks to determine whether the polymorphism has an effect on the pulmonary effectiveness of albuterol treatment. Because the cystic fibrosis population is routinely given albuterol, this study included cystic fibrosis patients to enhance the study’s clinical relevance.
More importantly, the bronchodilator response to beta-2 agonists shows high variability between individuals, prompting the investigation of a genetic component that may influence the effectiveness of albuterol.
CHAPTER 2: SUMMARY OF DISSERTATION RESEARCH

The specific details of this dissertation are presented in three appended papers; this chapter summarizes the research within the three appendices.

Chapter 2.1: Summary of Appendix A-Genetic Variation of Phenylethanolamine N-Methyltransferase Influences Circulating Catecholamines and Cardiovascular Function in Healthy Humans.

The study included in the first appendix aims to determine whether genetic variation of the phenylethanolamine N-methyltransferase (PNMT) enzyme at position -182 affects sympathetic activity and blood pressure regulation in healthy humans at rest. Because the cardiovascular system, sympathetic nervous system, and the renal system play a role in the regulation of blood pressure, we took measurements from these three systems with the aim of determining which ones were affecting blood pressure variation, if any.

Twenty normotensive adults were recruited and stratified accorded to homozygous genotype at PNMT position -182 (AA or GG genotype). A venous blood draw was taken to assess catecholamines (epinephrine and norepinephrine), sodium (Na\(^+\)) and creatinine in serum. A 24-hour urine collection was used to determine urine Na\(^+\) and creatinine. Measures of creatinine and Na\(^+\) in both urine and plasma were used to calculate fractional excretion of sodium with the following formula: \( FE_{Na} = \frac{(Urine \ Na^+ \times Plasma \ Creatinine)}{(Plasma \ Na^+ \times Urine \ Creatinine)} \). Measures of resting cardiac output (Q), heart rate, and blood
pressure (systolic, SBP, and diastolic, DBP) were taken in triplicate. Mean arterial pressure (MAP) was calculated from the measurements of systolic and diastolic blood pressure using the following formula: MAP = DBP + 1/3(SBP-DBP).

Systemic vascular resistance (SVR) was calculated using MAP and the reported average of central venous pressure of 5mmHg: SVR = (MAP - Central Venous Pressure* 80)/Q. Statistical comparisons between the AA and GG genotypes consisted of a t-test with a significance set at an α level of 0.05.

This study found that the GG group had higher circulating epinephrine levels compared to the AA group. Furthermore, the epinephrine to norepinephrine ratio was higher in the GG genotype group. These results suggest that the GG genotype confers a more functional form on the PNMT enzyme capable of converting more norepinephrine into epinephrine. The GG group exhibits elevated Q compared to the AA group, suggesting that the increased catecholamines in this group have a cardiac effect. Furthermore, the GG group had lower systemic vascular resistance. No differences in renal or blood pressure measurements (systolic, diastolic, MAP) were found between genotype groups.

Overall, the GG group had significantly higher circulating epinephrine, epinephrine: norepinephrine ratio, and cardiac output. Furthermore, this group had a significantly lower systemic vascular resistance. Overall, the results from this study suggest that the PNMT polymorphism at position -182 may have a sympathetic and cardiovascular effect.
Chapter 2.2: Summary of Appendix B- Genetic Variation of the Beta-2 Adrenergic Receptor Influences the Cardiovascular Response to Albuterol.

The goal of the research in the second appendix is to determine whether genetic variation of the beta-2 adrenergic receptor (B2AR) at amino acid position 16 has an effect on the cardiovascular response (including blood pressure) to albuterol. Albuterol is primarily administered as a pulmonary therapeutic. However, because albuterol administration is accompanied by an increase in catecholamines, it is possible that the cardiovascular system’s B2ARs could be activated resulting in an elevated cardiovascular state.

Forty-two subjects were recruited and stratified according to genotype at B2AR position 16 (AA, AG, or GG genotype). Cardiovascular measurements were taken at baseline (i.e. prior to albuterol administration); these included heart rate, cardiac output, and blood pressure (systolic and diastolic) measurements in triplicate. Albuterol, in the form of albuterol sulfate, was administered to subjects following baseline measures using a nebulizer. Cardiovascular measurements were taken again 60 minutes post-albuterol. MAP and SVR were calculated at baseline and 60-minutes post-albuterol as discussed in the previous section (Chapter 2.1). Prior to statistical testing, percent changes from baseline to 60-minutes post-albuterol were calculated for each genotype group. Statistical comparisons between the AA, AG, and GG genotypes were done using ANOVA testing with a significance set at an $\alpha$ level of 0.05.
This study found that the AG group exhibited the highest drop in SVR in response to albuterol administration; in contrast, the AA group exhibited an increase in SVR. There were no significant changes in cardiac output, heart rate, or blood pressure measurements (systolic, diastolic, MAP) between groups.

Overall, this study suggests that genetic variation of the B2AR at amino acid position 16 may influence the cardiovascular system, with the effect specifically occurring at the level of the vasculature.

Chapter 2.3: Summary of Appendix C- Genetic Variation of the Beta-2 Adrenergic Receptor and the Bronchodilator Response to Albuterol in Cystic Fibrosis.

The aim of the study in the final appendix is to determine whether genetic variation of the beta-2 adrenergic receptor has an effect on the airway response to albuterol in healthy controls and patients with cystic fibrosis (CF). Albuterol, a beta-2 agonist, binds to pulmonary B2ARs to cause bronchodilation and lung-fluid clearance. Evidence confirms the functional relevance of the B2AR polymorphism at amino acid position 16 but there is a lack of studies investigating the role of the polymorphism in the pulmonary response to beta-agonists. This study aims to address this gap in the literature. Furthermore, the study included cystic fibrosis patients because albuterol is a common CF therapeutic.

Thirty-one healthy and seventeen CF subjects were recruited for the study and stratified according to genotype. Subjects were part of a glycine-containing
(AG/GG) or a non-glycine containing group (AA). Pulmonary function testing maneuvers were done at baseline (i.e. prior to albuterol administration). Albuterol, in the form of albuterol sulfate, was administered to subjects following baseline measures using a nebulizer. Pulmonary function testing was done at 30-, 60-, and 90- minutes post-albuterol administration. Forced vital capacity (FVC), forced expiratory volume after one second (FEV₁), forced expiratory flow at 50% of the FVC (FEF₅₀) and forced expiratory flow rate at 25-75% of the forced vital capacity (MMF) were calculated. Percent changes from baseline to each timepoint (30-, 60-, and 90- minutes post-albuterol) were calculated for each genotype group. Statistical comparisons between genotypes for both subject groups (healthy and CF) were performed using t-test with a significance level set at an α level of 0.05.

This study found that percentage change of forced vital capacity from baseline to 30 minutes post-albuterol administration differs between genotypes. CF subjects with a glycine-containing genotype showed a larger percent increase in FVC 30 minutes following albuterol administration as compared to arginine homozygotes. No significant differences in percent changes in FEV₁, MMF, and FEF₅₀ from baseline to 30 minutes post-albuterol were found between genotypes within the CF subjects. Percent changes for all respiratory measures from baseline to 60 minutes and 90 minutes post-albuterol were similar between genotypes within the CF subject group. No significant differences in percent change for any respiratory measure at any time point (30, 60, nor 90 minutes) were found between genotypes within the healthy subject group.
Overall, this study suggests that genetic variation of the B2AR at amino acid position 16 may have an influence on the respiratory response to albuterol in CF patients. However, genetic variation of the B2AR did not seem to affect the response in healthy adults. This and similar studies could begin to elucidate the variability in response to albuterol often encountered in clinical settings.
CHAPTER 3: CONCLUSIONS AND FUTURE AIMS

3.1 Conclusions-Appendix A: Genetic Variation of Phenylethanolamine N-Methyltransferase Influences Circulating Catecholamines and Cardiovascular Function in Healthy Humans.

The aim of the first sub-study included in this dissertation, as summarized in the previous chapter, is to determine the influence of the polymorphism at position -182 of the phenylethanolamine N-methyltransferase (PNMT) enzyme on sympathetic and cardiovascular regulation. Twenty healthy adults were stratified according to PNMT genotype at position -182 (AA or GG genotype). Sympathetic, cardiovascular, and renal measurements were taken in order to identify what system could be affecting blood pressure. This study collectively demonstrated that genetic variation of the PNMT enzyme and the beta-2 adrenergic receptor may influence circulating catecholamines levels and cardiovascular function.

Specifically, this study showed that genetic variation of the PNMT enzyme at position -182 may influence circulating catecholamine levels, cardiac output and systemic vascular resistance. Elevated venous epinephrine and epinephrine:norepinephrine ratio in the GG genotype group could be driving the elevated cardiac output and the lower systemic vascular resistance also seen in this group, as compared to the AA genotype group. Cardiac output and systemic vascular resistance are both adrenergically regulated, and are thus sensitive to levels of circulating catecholamines.
The phenylethanolamine N-methyltransferase enzyme converts norepinephrine into epinephrine by transferring a methyl group to norepinephrine’s amino group. Circulating epinephrine elicits a variety of physiological changes due to its affinity to adrenergic receptors, which have a diverse distribution in the human body. Epinephrine, however, has a higher affinity for beta-adrenergic over alpha–adrenergic receptors. Furthermore, epinephrine has a higher affinity for beta 2-adrenergic receptors rather than the beta 1 counterparts.

Beta-adrenergic receptors, located in the heart, vasculature, and the kidney, mediate diverse functions when they are bound by catecholamines, the products of PNMT. These functions include chronotropy, inotropy, and vasodilation/vasoconstriction (in the systemic and also renal circulation); binding of epinephrine with beta-adrenergic receptors can therefore illicit physiological effects that can mediate increases in blood pressure, either temporary or in a more permanent manner, as is the case in hypertension. In fact, elevations of PNMT, which makes epinephrine, have been documented in hypertension. Further highlighting the link between PNMT and blood pressure, hypertensive animal models display amelioration of high blood pressure when given a PNMT enzyme inhibitor.

This study suggests that the PNMT polymorphism at position -182 influences sympathetic and cardiovascular mediators of blood pressure changes but does not influence blood pressure regulation itself. However, the clinical relevance of this study cannot be denied. This study’s finding that the GG
genotype displays higher circulating epinephrine and norepinephrine:epinephrine ratio has implication on clinical states displaying elevated sympathetic drive such as heart failure and phaeochromocytoma.

Chapter 3.2: Conclusions-Appendix B: Genetic Variation of the Beta-2 Adrenergic Receptor Influences the Cardiovascular Response to Albuterol.

The aim of the second sub-study included in this dissertation, as summarized in the previous chapter, is to determine the influence of the polymorphism at amino acid position 16 of the beta-2 adrenergic receptor (B2AR) on the cardiovascular response to the administration of albuterol. Forty-two healthy adults were stratified according to B2AR genotype at amino acid position 16 (AA, AG, or GG genotype). Cardiovascular measurements, including heart rate, stroke volume, cardiac output, blood pressure, and calculations of mean arterial pressure and systemic vascular resistance were used in order to identify whether albuterol administration caused cardiovascular alterations, by genotype.

This study found that genetic variation of the B2AR at amino acid position 16 may influence the change in systemic vascular resistance following the administration of albuterol. Because administration of nebulized albuterol is accompanied by an increase in catecholamines, cardiovascular deviations from baseline is likely because the cardiovascular system is sympathetically regulated. In this study, an elevation in catecholamines may be differentially affecting vascular B2ARs in subjects with the GG genotype, leading to a significant drop in
systemic vascular resistance. No differences in other cardiac measures including as cardiac output, however, were seen between subject groups.

The B2AR is a classical G-protein coupled receptor found in the heart and vasculature and mediates sympathetically-induced alterations in cardiovascular function. Interaction between catecholamines and the B2AR lead to increases in cAMP and PKA which act as mediators to these cardiovascular changes. In the heart, PKA phosphorylates L-type calcium channels, inducing increases in cytosolic calcium and ultimately increases in cardiac contractility. In the vasculature, increases in cAMP from B2AR stimulation lead to inhibition of myosin light chain kinase, resulting in increased vasodilation.

Nebulized albuterol is a common clinical therapeutic in respiratory conditions such as asthma and cystic fibrosis. A study in our laboratory has shown that administration of albuterol is accompanied by increases in circulating catecholamines; albuterol, therefore, poses the risk of increased heart rate, stroke volume, and changes in systemic vascular resistance, all of which are unwanted side effects. This study identified that the AG genotype of the B2AR polymorphism at position 16 displays the largest change in systemic vascular resistance in response to nebulized albuterol. The rest of the cardiovascular measurements did not yield significant results; therefore, this study is unable to clearly identify which genotype displays the largest change in cardiovascular function as a response to albuterol administration. An expansion of this study is needed for clearer results.
Chapter 3.3: Conclusions-Appendix C: Genetic Variation of the Beta-2 Adrenergic Receptor and the Bronchodilator Response to Albuterol in Cystic Fibrosis.

The aim of the third and final sub-study included in this dissertation, as summarized in the previous chapter, is to determine the influence of the polymorphism at amino acid position 16 of the beta-2 adrenergic receptor (B2AR) on the pulmonary response to the administration of the beta-2 agonist albuterol. Thirty-one healthy adults and seventeen cystic fibrosis patients were stratified according to B2AR genotype; this study included subjects with cystic fibrosis (CF) because albuterol is a common therapeutic for this disease population. Subjects were placed in either a non-glycine containing (AA) or a glycine-containing group (AG/GG). Pulmonary function testing was done at 30-, 60-, and 90-minutes post-albuterol administration.

This study found that the polymorphism at position 16 may influence the pulmonary response to albuterol. CF subjects with glycine-containing genotypes had a greater increase in forced vital capacity 30 minutes following albuterol administration. The polymorphism did not seem to influence the other measured respiratory parameters (FEV₁, FEF₅₀, MMF) in CF subjects at the 30 minute timepoint. No genotypic differences in pulmonary function after albuterol administration were seen in healthy subjects at any time points.

Albuterol, a beta-2 agonist, binds to B2ARs in the lungs causing bronchodilation by way of pulmonary smooth muscle relaxation but also lung fluid
clearance through the phosphorylation of the epithelial sodium channel, the
cystic fibrosis transmembrane conductance regulator and the sodium-potassium
ATPase. These pulmonary changes, similar to the cardiovascular changes due to
B2AR stimulation, are mediated by increases in PKA.

This study seems to suggest that the GG genotype at B2AR position 16
exhibits elevated pulmonary responsiveness to albuterol; there results, however,
are not entirely conclusive, considering that most pulmonary parameters did not
yield significant results. Despite lack of clarity, these results show that the GG
genotype is more functional compared to the other genotypes, an idea that is
pervasive in the literature.

Chapter 3.4: Limitations and Future Aims

In the future, several limitations of this study need to be addressed.
Sympathetic nervous system activity was assessed indirectly in the PNMT study
(Appendix A) via venous catecholamine measurements. Measurement of arterial
catecholamine levels or muscle sympathetic nerve activity via microneurography
more accurately measures sympathetic activity. The studies in appendix A and
B (the PNMT study and beta-2/albuterol study, respectively) could benefit from
more accurate blood pressure measurements. The auscultation technique was
used in these studies, employing the use of a traditional blood pressure cuff and
three consecutive blood pressure measurements. Twenty-four hour blood
pressure monitoring is a more accurate gauge of blood pressure regulation
compared to isolated measurements during a laboratory visit which could be
subject to the “white-coat” effect. Finally, all three studies could be improved by increasing subject recruitment; the results that neared significance especially could perhaps gain clarity with more robust subject numbers in the genotype groups. Furthermore, more recruitment would allow investigation of the heterozygote genotype in all studies.

This study addresses the lack of human mechanistic studies in exploring genetic variation’s possible role in physiological function. However, the influence of other genetic variants on cardiopulmonary regulation and the sympathetic nervous system cannot be ignored, including those within the genes encoding the PNMT enzyme and the B2AR but also others. As proposed by George Pickering, the collective effect of multiple gene variants, each with a small yet significant effect, is what ultimately influences physiological function. A multitude of genes relevant to cardiopulmonary and sympathetic regulation exist and cannot be ignored, including genes in relevant signaling pathways but also in non-related pathways. For example, genetic variation of chromogranin B, a matrix protein of catecholamine storage vesicles, is associated with alterations in catecholamine secretion and blood pressure. Genetic variation of the beta-1 adrenergic receptor as well has been implicated in hypertension. A broad exploration of multiple genes is necessary to evaluate the effect of genetic variation on any single physiological function. This is especially the case in essential hypertension, whose complex etiology disallows the identification of a single cause for the condition.
Epinephrine, the product of the PNMT enzyme, binds to the B2AR to modulate cardiovascular function. Because the PNMT enzyme and the B2AR have a direct relationship, it is necessary to explore the combined effect of the PNMT polymorphism at position -182 and the B2AR polymorphism at amino acid position 16. Perhaps the cumulative effect of these two genetic variants is stronger and more relevant than the individual effects of these polymorphisms. Finally, considering previous research on the relevancy of the PNMT enzyme and the B2AR in blood pressure regulation, it is possible that these variants have a stronger effect in a hypertensive state with muted effects in normotensive individuals. Future research could address this and the other concerns detailed above.

The results of this study implicate genetic variation of the phenylethanolamine N-methyltransferase enzyme and the beta-2 adrenergic receptor to both cardiopulmonary and sympathetic regulation. Therefore, several clinical conditions could benefit from these studies’ findings. Phaeochromocytoma, a tumor of the adrenal medulla, results in dangerous elevations in blood pressure due to elevations in circulating catecholamines. The results found in this dissertation therefore would suggest that pheochromocytoma patients with the GG genotype at PNMT position -182 would exhibit higher elevations in blood pressure; this theoretically would be a result of higher epinephrine levels seen in this genotype at baseline. The results of this study would also suggest that carriers of the GG genotype, considering the higher baseline epinephrine and cardiac output, could exhibit increased performance
during exercise. Heart failure is another condition that is relevant to the studies presented in this dissertation. An increase in catecholamines accompanies heart failure; though this occurs initially as a mechanism to restore cardiac output, catecholamines ultimately are cardiotoxic and initiate pathogenic cardiac remodeling\textsuperscript{82,83}. The GG genotype at position -182 of the PNMT enzyme would likely be detrimental in heart failure, despite its advantage at initial stages of heart failure. Furthermore, albuterol is often used in heart failure to alleviate deteriorations in pulmonary function such as airway obstruction and diffusion impairments\textsuperscript{84-87}. Results of this study suggest that carriers of the GG genotype at amino acid position 16 of the beta-2 adrenergic receptor would most benefit from albuterol administration for treatment of pulmonary complications of heart failure, leading to a possible improvement in quality of life but also in exercise performance.
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APPENDIX A: GENETIC VARIATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE INFLUENCES CIRCULATING CATECHOLAMINES AND CARDIOVASCULAR FUNCTION IN HEALTHY HUMANS

Paper is currently under review for publication in the journal of Applied Physiology, Nutrition, and Metabolism.

Abstract
Susceptibility to hypertension can be influenced by variation in genes that alter cardiovascular function, renal function, or both. Previous work has demonstrated that the gene encoding phenylethanolamine-N-methyltransferase (PNMT) is a candidate gene for hypertension. We sought to determine the influence of genetic variation of PNMT (A-182G) on renal Na⁺ handling and cardiovascular function in humans. We collected epinephrine (Epi), norepinephrine (NE), Na⁺, and creatinine from serum in 20 normotensive subjects (n: AA=6, GG=14). We also performed 24-hour urine collections to assess urine Na⁺ and creatinine and measured cardiac output (Q), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). We then calculated fractional excretion of Na⁺ (FE₇ₙₐ), stroke volume (SV), systemic vascular resistance (SVR), and mean arterial blood pressure (MAP). The GG genotype had higher Epi and Epi/NE ratio, but no difference in NE when compared to AA (Epi=98±21 vs. 53±10 pg/L; Epi/NE ratio= 0.31±0.07 vs. 0.13±0.03; NE= 344±56 vs. .462±79pg/L; GG and AA, respectively; mean±SE, p<0.05 for Epi and Epi/NE ratio). We found no differences between genotypes in 24-hour renal Na⁺ handling (serum Na⁺, urine Na⁺, or FE₇ₙₐ), HR, SBP, DBP or SV between the groups. However, the GG group
had a higher Q and lower SVR when compared to the AA group (Q= 6.0±0.5 vs. 4.8±0.4L/min; SVR= 1020±64 vs. 1316±95 dynes*sec/cm$^5$, for GG and AA, respectively mean±SE, p<0.05). These results suggest genetic variation of $PNMT$ may influence cardiac output, but exerts minimal influence on renal Na$^+$ handling or blood pressure in normotensives.

**Introduction**

Blood pressure regulation is multifactorial, primarily involving renal control of sodium handling and fluid reabsorption, regulation of vascular and cardiac function, and the autonomic nervous system$^{4,5}$. Despite the prevalence of a renocentric view of long-term blood pressure regulation, an increasing amount of research stresses the role of the sympathetic nervous system in hypertension$^{74-76}$. Phenylethanolamine N-methyltransferase (PNMT) catalyzes the conversion of norepinephrine to epinephrine and has a vital role in mediating sympathetic responses$^{39,40}$. This enzyme is principally found in the adrenal medulla, brain stem, retina, and heart$^{41,42}$. Epinephrine, the product of PNMT, affects blood pressure regulation through both its renal and cardiovascular effects. In the kidney, epinephrine binds to renal $\alpha_1$ adrenergic receptors causing vasoconstriction, renin release, and enhanced Na$^+$ reabsorption, which can lead to blood pressure elevations$^{77}$. Epinephrine also modifies vascular tone and causes increases in heart rate and cardiac contractility$^{78,79}$.

The gene for PNMT ($PNMT$) is found on human chromosome 17 (17q21-q22), and its specific location constitutes a gene region that has been linked to high blood pressure$^{34,35}$. The phenylethanolamine N-methyltransferase enzyme
has also been associated with elevations in blood pressure. Animal models of hypertension, including the Dahl salt-sensitive rat and the spontaneously hypertensive rat (SHR), demonstrate elevated PNMT activity\(^ {80, 81 82}\). Administration of SKF 7698, a PNMT inhibitor drug, resulted in significant decreases in blood pressure in Doca-salt hypertensive rats\(^ {80}\). However, it is unclear whether this decrease was due to PNMT inhibition or by SKF 7698’s alpha-adrenergic receptor blockade property, which would block the vasoconstricting effect of the alpha adrenergic receptors. Furthermore, catecholamine elevations, as seen in pheochromocytomas, are associated with a hypertensive state, further stressing the link between catecholamines and blood pressure regulation\(^ {83, 84}\).

Various functional polymorphisms for \textit{PNMT} have been identified, including an adenine (A) to guanine (G) substitution at position -182 (rs#876493) of the promoter region; this polymorphism has a minor allele frequency of 30% in whites and 70% in blacks\(^ {50}\). The A allele at position -182 has been associated with lower urine epinephrine and decreased in-hospital mortality from acute kidney injury\(^ {51}\). Additionally, the \textit{PNMT} polymorphism at position -182 was associated with differential diastolic blood pressure in men versus women, with women overall showing decreased diastolic blood pressures for the three genotypes tested (GG, AG, AA)\(^ {85}\). Furthermore, position -182 of \textit{PNMT} has been shown to affect blood pressure in African Americans, with the G allele tending to be more prevalent in hypertensives\(^ {52}\). Most recently, the GG genotype at \textit{PNMT} position -182 was associated with higher resting venous norepinephrine; the
same study also associated this polymorphism to the change in venous norepinephrine in response to exercise. Though these previous studies discuss the same single nucleotide polymorphism (SNP), the SNP number used throughout the literature is not uniform; however, all these studies refer to the same PNMT polymorphism, as verified by identical reference SNP (rs) numbers. As such, our study refers to this polymorphism as the PNMT polymorphism as position -182 which is in-line with more recent published studies for this polymorphism. Other polymorphisms within the PNMT gene have been identified and correlated to hypertension, further strengthening the argument that the PNMT gene plays a strong role in blood pressure regulation.

Little work has been done to address the mechanism by which PNMT variants may alter the regulation of blood pressure. Based on these previous studies, we sought to determine whether genetic variation at position -182 affects blood pressure and, furthermore, to determine whether PMNT genotype-mediated differences in blood pressure could be attributed to differences in renal sodium handling or cardiovascular function. We predicted that individuals with the G allele at position -182 of the PNMT gene would exhibit increases in circulating epinephrine levels. Furthermore, we predicted that the elevated epinephrine of the GG group would translate to elevated cardiovascular and renal parameters compared to the AA group due to epinephrine’s action on the heart, vasculature, and kidney.
Methods

Subjects

Twenty healthy normotensive subjects were recruited for the study. The protocol was reviewed and approved by the University of Arizona Institutional Review Board and all aspects of the study were performed according to the latest revision of the Declaration of Helsinki. All participants provided written informed consent prior to study.

Protocol

Subjects arrived to the laboratory in a fasted state. During the first laboratory visit, subjects underwent screening consisting of a complete blood count, a maximal exercise test and pregnancy test (in females) to rule out kidney dysfunction and/or anemia, cardiopulmonary abnormalities and pregnancy, respectively. Smoking was also considered an exclusion criterion. Peak $\text{O}_2$ consumption was determined via a maximal exercise test on a cycle ergometer. Oxygen consumption and carbon dioxide production ($\text{VO}_2$ and $\text{VCO}_2$), respiratory exchange ratio (RER), $\text{SaO}_2$, and heart rate (HR) were continuously monitored during testing. The protocol consisted of starting at an initial resistance workload that was increased in a stepwise manner throughout the duration of the test. Peak $\text{VO}_2$ was the $\text{VO}_2$ measure taken immediately prior to end of test. Testing was ended when RER became greater than 1.15, the subject could no longer maintain a pedal rate of >60RPM, the subject’s rating of perceived exertion on the Borg scale reached 18 out of 20, HR was >100% predicted, or $\text{SaO}_2$ dropped
below 85%. Gas exchange during the maximal exercise test was assessed with a Medical Graphics CPX/D (St. Paul, MN) metabolic cart interfaced with a Perkin Elmer MGA-1100 mass spectrometer (Wesley, MA). At the end of the screening visit, subjects were given a urine collection container and were instructed to collect urine for 24 hours prior to their second laboratory visit. On the study visit (second visit), subjects again arrived in a three-hour fasted state (including no caffeine intake) and had blood drawn for a renal function panel (serum Na⁺, K⁺, and creatinine), catecholamines, and a complete blood count. Subjects were instructed to rest quietly for 20 minutes prior to the blood draw. Following the blood draw, cardiac output (Q) was measured in triplicate, with sufficient time between measures to allow wash-out of test gases. Blood pressures were also measured in triplicate following determination of Q. Both cardiac output and blood pressure measures were taken in a seated position.

PNMT genotyping

Genotyping was performed on genomic DNA extracted from buccal swabs using a custom magnetic bead protocol using SILANE chemistry (Life Technologies, Carlsbad, CA) on BioSprint 96 (QIAGEN, Gaithersburg, MD). Samples were incubated at 55°C for 4-8 hours with Proteinase K prior to extraction. DNA quantitation was performed using PicoGreen (Life Technologies) and all samples were subsequently normalized to a final concentration of 5 ng/µl. Pre-validated primers and probes for the TaqMan Allelic Discrimination Assay were obtained from Life Technologies (rs#876493). Reactions were set up in 10µL on 384-well plates using TaqMan Universal PCR Master Mix, No
AmpErase® UNG (Life Technologies) with 10ng DNA, and 1X Assay Mix, including one no template control (NTC) and one blank containing no mastermix. The thermal cycling reactions (95 °C for 10 minutes, 50 cycles of 92 °C for 15 seconds and 60 °C for 1 minute) were analyzed on a 7900 Real-Time PCR System (Life Technologies) including a pre-read protocol for removal of background fluorescence run at 60 °C for 1 min. All samples were analyzed with Applied Biosystems Genotyper software (SDS system, version 2.3).

Assessment of Urine and Plasma Na\textsuperscript{+} and Creatinine

Urine Na\textsuperscript{+} and creatinine (Cr) levels were determined with a 24-hour collection prior to the second study visit. Plasma Na\textsuperscript{+} and Cr concentrations were determined from blood sampling through a venous catheter. Subjects were instructed to sit quietly for 20 minutes before the blood draw. Both urine and serum ion levels were assessed using ion-selective electrodes at the University of Arizona Medical Center Pathology Laboratory.

Calculation of Fractional Excretion of Sodium

The fractional excretion of Na\textsuperscript{+} (FE\textsubscript{Na}) was calculated as a means of assessing renal Na\textsuperscript{+} and water handling. The equation used is as follows: \( \text{FE}_{\text{Na}} = \frac{(\text{Urine Na}\textsuperscript{+} \times \text{Plasma creatinine})}{(\text{Plasma Na}\textsuperscript{+} \times \text{Urine Creatinine})}. \)
Assessment of Cardiovascular Function and SVR

Upon arrival to our laboratory subjects were placed on a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) to monitor heart rate. Cardiac output (Q) was assessed using a previously validated 8-10-breath acetylene (C₂H₂) rebreathe technique using a 5-liter rebreathe bag containing 0.6% C₂H₂, 9% He, and 35% O₂. Briefly, a pneumotachograph was connected to a non-rebreathing Y valve (Hans Rudolph, KC, MO) with the inspiratory port connected to a pneumatic switching valve that allowed for rapid switching from room air to the test gas mixture. Gases were sampled using a mass spectrometer (Perkin-Elmer) integrated with custom analysis software for the assessment of Q. Consistent bag volumes were assured using a timed switching circuit which, given a consistent flow rate from the tank, resulted in the desired volume of gas. The switching circuit and tank system were checked prior to each test for accurate volumes. At the end of a normal expiration, the subjects were switched into the rebreathe bag and instructed to nearly empty the bag with each breath for 8-10 consecutive breaths. Following each maneuver, the rebreathe bag was emptied with a vacuum and refilled immediately prior to the next maneuver. Cardiac output was measured three times per subject with enough time between each measure to allow wash-out of test gases. At the start of each maneuver, there was no residual gas in the dead space of the apparatus, nor from the exhaled air from the subjects, as determined through gas sampling with the mass spectrometer. The average of the three measures was determined for each subject and used in analysis. The reported heart rate average was
calculated from heart rates taken during determination of cardiac output (when subjects were breathing from the rebreathe bag). Stroke volume was calculated from Q and HR (SV = Q/HR \times 1000).

Blood pressure was assessed using the auscultation technique with subjects in a seated position with the same technician performing all measures. Mean arterial blood pressure (MAP) was calculated using the equation: MAP = DBP + 1/3(SBP-DBP), where DBP is diastolic blood pressure and SBP is systolic blood pressure. Systemic vascular resistance was calculated using the following formula: SVR = (MAP - Central Venous Pressure \times 80)/Q, where central venous pressure is assumed to be five mmHg.

Statistical Analysis

All statistical comparisons were performed using the SPSS statistical software package (v. 18.0; SPSS, Inc., Chicago, IL). Mean values for all parameters were calculated as an average and were presented in the mean ± standard error format unless otherwise stated. After confirming equality of variance with a Levene’s test, comparisons between genotype groups were performed using an independent samples t-test with significance set at an α level of 0.05.
Results

Subject characteristics

Twenty normotensive individuals completed the study (subject demographics, Table 1). Six subjects were homozygous for the A allele at position -182 (AA genotype group) and fourteen were homozygous for the G allele (GG genotype group). No significant demographic differences existed between genotype groups (age, height, weight, body mass index, body surface area, and peak VO$_2$) with the exception of gender, with 50% of the GG group consisting of women versus the AA group being 20% female. With the sample size of the AA group being six subjects, 20% female constitutes only one individual. Furthermore, there were no significant differences in alcohol consumption (AA=6.7±2.0, GG=7.2±3.1 drinks/week) or exercise habits (AA=8.4±2.1, GG=6.4±1.0 hours/week) between genotype groups.

Catecholamines

The GG group exhibited higher plasma epinephrine when compared to the AA genotype group (Epi=98±21 vs. 53±10 pg/L, p<0.05) (Figure 1). However, there was no difference between groups in regards to the amount of norepinephrine in plasma (NE= 344±56 for GG, 462±79pg/L for AA) (Figure 2). The GG genotype group had a higher Epi/NE ratio than the AA group (Epi/NE= 0.31±0.07 vs. 0.13±0.03, p<0.05) (Figure 3).
Cardiovascular and renal measures

Heart rate and stroke volume, contributors to cardiac output, were not significantly different between groups (Table 2). However, cardiac output was significantly greater in the GG group ($Q = 6.0 \pm 0.5$ vs. $4.8 \pm 0.4$ L/min, GG vs. AA) (Figure 4). The GG group showed a trend towards lower diastolic blood pressure and mean arterial pressure but these measures, along with systolic blood pressure, were not significantly different between groups (DBP = $70 \pm 3$ vs. $75 \pm 2$ mmHg; MAP = $83 \pm 3$ vs. $87 \pm 2$ mmHg; SBP = $109 \pm 4$ vs. $110 \pm 2$ mmHg; GG vs. AA) (Table 2). However, systemic vascular resistance was lower in the GG group (SVR = $1020 \pm 64$ vs. $1316 \pm 95$ dynes*sec/cm$^5$, GG vs. AA, p<0.05) (Figure 5).

None of the renal parameters measured were different between groups (Table 2). Serum Na$^+$, urine Na$^+$, and fractional excretion of sodium were similar between the GG and AA genotype groups (Serum Na$^+$: $138 \pm 0.3$ vs. $139 \pm 1$ mmol; 24-hour urine Na$^+$ = $98 \pm 14$ vs. $115 \pm 29$ mmol; FE$_{Na}$ = $0.6 \pm 0.1$ vs. $0.7 \pm 0.2$; GG vs. AA).

Comparison of heterozygote group

Eleven additional subjects with the heterozygotic condition (AG genotype) were recruited in the study; we sought to determine how this group compared to the two homozygous groups (AA and GG) and whether the heterozygote group was similar to either one of homozygous groups (AA and GG). We found that the AG
group was not similar to either homozygote group in neither demographics nor cardiovascular measurements (Table 3).

Discussion

In the present study we demonstrate that genetic variation of \textit{PNMT} at position -182 may influence cardiac output in normotensive individuals. Serum epinephrine levels and epinephrine to norepinephrine ratio were both significantly higher in the GG group when compared to the AA group. Serum norepinephrine was similar between both groups. Heart rate and stroke volume, determinants of cardiac output, were similar between groups. However, the GG genotype exhibited a statistically higher cardiac output compared to the AA group. We attribute the GG group’s higher Q to the group’s slightly higher SV as compared to the AA group. Furthermore, the GG group has a lower HR compared to the AA group, leading us to conclude that GG’s higher Q is being driven by SV. In contrast, the AA group’s lower Q is being driven by the group’s lower SV when compared to the GG group (and not heart rate since heart rate is higher in the AA group). The GG group exhibited a trend towards a lower DBP and MAP; the values did not reach significance likely due to the small sample size. Systolic blood pressure did not differ between genotypes. However, we found lower systemic vascular resistance, as determined by mean arterial pressure and cardiac output, in the GG group. We attribute the lower SVR in the GG genotype to the larger Q and lower MAP this group displays compared to the AA genotype group. More specifically, decreased SVR in the GG group could be an effect of this group’s elevated epinephrine. Theoretically, elevated epinephrine
would lead to an increased vasodilatory response in the GG group due to epinephrine’s binding to beta-2 adrenergic receptors; however, we found that epinephrine was not correlated with SVR. Additionally, there were no differences in 24-hour renal sodium handling (serum Na\(^+\), urine Na\(^+\), or FE\(_{Na}\)). The results of this study suggest that the GG genotype of the polymorphism at position -182 of \(PNMT\) exhibits differences in circulating epinephrine levels when compared to the AA genotype. Furthermore, we have noted that this difference in epinephrine quantity between genotypes appears to translate into differential cardiac responses, with no effect at the level of the kidney.

The A allele is the variant allele for the \(PNMT\) polymorphism at position -182; the G allele is considered the wild type condition. Oftentimes in polymorphism research, the heterozygote subjects are grouped within one of the homozygote groups based on physiological similarities as dictated by the variant allele. For example, subjects with the heterozygous AT genotype for the polymorphism of the epithelial sodium channel (ENaC) at amino acid 663 are often grouped with the homozygous TT group \(^{90,91}\). We wanted to explore whether the heterozygote group was similar to either of the homozygote groups for the \(PNMT\) polymorphism at position -182. We determined that the AG heterozygote group was similar to both homozygous groups. Therefore, this prevents grouping subjects by variant (i.e. forming a AA/AG group) or otherwise. In fact, grouping subjects by variant (variant-containing vs. no variant containing groups) did not yield significant differences in any of the reported measurements (Table 4). We conclude that either this study has to be expanded in order to
make differences between genotypes more explicit or that the heterozygote group should not be considered a group that behaves in a singularly different manner as compared to the homozygote groups.

Phenylethanolamine N-methyltransferase catalyzes the last step in epinephrine formation by facilitating the transfer of a methyl group from S-adenosyl-L-methionine to the amino group of norepinephrine. Epinephrine has a complex pattern of effect, with a higher affinity for β-adrenergic over α-adrenergic receptors and overall greater affinity for β₂ adrenergic receptors over β₁. Therefore, epinephrine can cause increases in heart rate and contractility by binding to cardiac β₁ and β₂ receptors, and change vascular tone by binding to α₁ or β₂ vascular receptors. In the kidney, epinephrine mediates vasoconstriction of renal arteries decreasing glomerular filtration rate, stimulates renin release, hence facilitating increased sodium reabsorption/retention. Therefore, epinephrine’s mechanism of action involves physiological events that possibly can play a part in development of hypertension.

Of interest, our study demonstrates that genetic variation of PNMT results in differences in circulating epinephrine and also the epinephrine/norepinephrine ratio. Although we found that this alteration in catecholamines primarily influenced cardiac function, one could see how this variation could be of particular importance to patients with elevated sympathetic drive, such as heart failure. Multiple studies have investigated other polymorphisms of the PNMT gene and, similar to our study, have confirmed their physiological relevance. For the polymorphism at intronic position -280, the GG genotype was associated with
greater circulating epinephrine and epinephrine to norepinephrine ratio in response to exercise. A transfection study involving COS cells confirmed that two other SNPs resulting in amino acid changes, Asn9Ser and Thr98Ala, have also been shown to exhibit differential enzyme activity and quantity.

There are various explanations as to why polymorphisms of the PNMT gene lead to differential enzymatic activity. One common theory is that polymorphisms alter the transcription of the gene itself. For example, the polymorphism at position -182 has been shown to interrupt the binding of transcription factor SOX17, with the G allele displaying increased binding. Increased SOX17 binding could explain the increased circulating epinephrine of the GG group in this present study. This stresses the possibility that polymorphisms can affect transcriptional rates; however, other mechanisms of enhanced PNMT activity exist. One study in particular showed that the Asn9Ser and Thr98Ala PNMT polymorphisms had an effect on enzyme quantity and activity due to elevated proteosome-mediated degradation in these variants.

Elevations in PNMT activity have been implicated in hypertension, suggesting the possibility of PNMT inhibition as a method of ameliorating high blood pressure. However, PNMT inhibitors used in animal research have proved to be toxic, or lack the efficacy and specificity necessary for human use. Because genetic variation of PNMT does alter epinephrine and epinephrine/norepinephrine levels, it may be important to tailor an individual's therapy (i.e. beta-blockers, etc.) according to their PNMT genetics, although much larger studies are certainly needed to determine this.
Limitations

Our study has a relatively small sample size, including only 20 subjects. Previous research investigating whether the PNMT polymorphism at position -182 affects blood pressure shows conflicting results; larger sample sizes are necessary in order to concretely establish whether blood pressure is influenced by this polymorphism. Despite this limitation of sample size, our study is the only study, to date, to investigate the mechanistic basis of PNMT gene variation’s possible influence on blood pressure by assessing its influence on the cardiovascular system and the renal system.

Other limitations include our use of manual blood pressure measurements via a sphygmomanometer. This type of blood pressure measurement is not as accurate as intra-arterial blood pressure monitoring. Another caveat in our study is the use of venous catecholamines rather than arterial catecholamine collection. Furthermore, we used normotensive subjects rather than hypertensive. It is possible that PNMT gene variation at position -182 is more relevant in a hypertensive population rather than one with controlled blood pressure. Along with larger sample sizes, further studies on the relationship between PNMT gene variation at position -182 and blood pressure need to include intra-arterial blood pressure measurements, catecholamines determined from arterial blood, and investigation of this polymorphism in hypertensive subjects. Furthermore, we are currently unable to measure central venous pressure; we use the reported average of 5mmHg to calculate systemic vascular resistance. Lastly, the authors recognize that the acetylene technique for
determination of cardiac output is oftentimes not thought to be accurate at rest. However, this technique results in values of resting cardiac output similar to values determined by echocardiography\textsuperscript{107}. Furthermore, the acetylene technique has been used to determine resting cardiac output in response to fluid loading and the technique has demonstrated enough sensitivity to determine changes at rest\textsuperscript{69}.

**Conclusions**

In summary, we found that genetic variation of \textit{PNMT} at position -182 may influence venous catecholamines, cardiac output, and systemic vascular resistance. Our results suggest that the SNP at position -182 does not affect renal Na\textsuperscript{+} handling or blood pressure regulation but we studied a relatively small amount of subjects for each genotype group. Another recent study had similar findings to our results and did not find an association between this SNP and hypertension\textsuperscript{108}. We propose that perhaps this polymorphism has a stronger relevance within a hypertensive population rather than a healthy population like the one tested in our study. It is also possible that other PNMT gene variants have a stronger role in blood pressure regulation. Further mechanistic studies in hypertension patients will help further elucidate the role of \textit{PNMT} polymorphisms in cardiovascular function, renal function, and blood pressure regulation.
### Table 1. Subject Characteristics

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>50*</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24±6</td>
<td>29±8</td>
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<tr>
<td>Height (cm)</td>
<td>171±8</td>
<td>174±12</td>
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<tr>
<td>Weight (Kg)</td>
<td>68±12</td>
<td>70±10</td>
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<tr>
<td>BMI (kg/m²)</td>
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<td>23±4</td>
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<tr>
<td>BSA (m²)</td>
<td>1.8±0.2</td>
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<tr>
<td>VO₂peak (% predicted)</td>
<td>92±35</td>
<td>118±27</td>
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</table>

Mean ± SD. BMI=Body mass index, BSA= Body surface area, VO₂peak= peak oxygen consumption, *p<0.05 between groups.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Heart rate (beats/minute)</td>
<td>78±3</td>
<td>82±10</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>75±7</td>
<td>71±10</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>109±4</td>
<td>110±2</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70±3</td>
<td>75±2</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>83±3</td>
<td>87±2</td>
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<tr>
<td>Serum Na(^+) (mmol)</td>
<td>138±0.3</td>
<td>139±1.0</td>
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<tr>
<td>24-hr Urine Na(^+) (mmol)</td>
<td>98±14</td>
<td>115±29</td>
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<tr>
<td>FE(_{Na})</td>
<td>0.6±0.1</td>
<td>0.7±0.2</td>
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Mean ± SE. FE\(_{Na}\) = fractional excretion of sodium.
Table 3: Heterozygote comparisons

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<td>Height (cm)</td>
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<td>174±4</td>
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<td>Weight (Kg)</td>
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<td>BSA (m²)</td>
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<td>Heart rate (beats/minute)</td>
<td>67±7</td>
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<td>Stroke volume (ml/beat)</td>
<td>78±11</td>
<td>68±5</td>
<td>78±5</td>
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<tr>
<td>Cardiac output (L/min)</td>
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<td>5±0.4</td>
<td>6±0.4</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>110±2</td>
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<td>109±4</td>
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<td>Diastolic blood pressure (mmHg)</td>
<td>75±2</td>
<td>72±2</td>
<td>70±3</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>87±2</td>
<td>84±2</td>
<td>83±3</td>
</tr>
<tr>
<td>SVR (dynes*sec/cm⁵)</td>
<td>1315±96</td>
<td>1196±100</td>
<td>1020±64</td>
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Table 4: Subject Grouping by Variant

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<tr>
<td>Heart rate (beats/minute)</td>
<td>74±3</td>
<td>74±3</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>72±5</td>
<td>78±5</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>5±0</td>
<td>6±0</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>109±2</td>
<td>109±4</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70±3</td>
<td>73±2</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>85±2</td>
<td>83±3</td>
</tr>
<tr>
<td>SVR (dynes*sec/cm$^5$)</td>
<td>1239±72</td>
<td>1020±65</td>
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<td>Epinephrine (pg/L)</td>
<td>55±10</td>
<td>58±11</td>
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<tr>
<td>Norepinephrine (pg/L)</td>
<td>311±20</td>
<td>266±25</td>
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Fig. 1 Plasma epinephrine levels in pg/L according to genotype group. The filled in bar represent the GG group (homozygous for guanine at PNMT position -182) and the open bar represents the AA group (homozygous for adenine at PNMT position -182). The error bars represent the SE of the mean. *P <0.05 between groups.
Fig. 2 Plasma norepinephrine levels in pg/L according to genotype group. The filled in bar represent the GG group (homozygous for guanine at PNMT position -182) and the open bar represents the AA group (homozygous for adenine at PNMT position -182). The error bars represent the SE of the mean. No significant difference was found between genotypes.
Fig. 3 Epinephrine to norepinephrine ratio according to genotype group. The filled in bar represent the GG group (homozygous for guanine at PNMT position -182) and the open bar represents the AA group (homozygous for adenine at PNMT position -182). The error bars represent the SE of the mean. *P <0.05 between genotypes.
**Figure 4**

*Fig. 4* Cardiac output (Q) in L/min according to genotype group. The filled in bar represent the GG group (homozygous for guanine at *PNMT* position -182) and the open bar represents the AA group (homozygous for adenine at *PNMT* position -182). The error bars represent the SE of the mean. *P <0.05 between genotypes.
**Figure 5**

![Bar chart showing systemic vascular resistance (dyne*sec/cm^5) for GG and AA genotypes of PNMT.](image)

**Fig. 5** Systemic vascular resistance in dyne*sec/cm^5^ according to genotype group. The filled in bar represent the GG group (homozygous for guanine at PNMT position -182) and the open bar represents the AA group (homozygous for adenine at PNMT position -182). The error bars represent the SE of the mean. *P <0.05 between genotypes.
References


30. Romero FA, Vodonick SM, Criscione KR, McLeish MJ, Grunewald GL. Inhibitors of phenylethanolamine n-methyltransferase that are predicted to penetrate the blood-brain barrier: Design, synthesis, and evaluation of 3-fluoromethyl-7-(n-substituted aminosulfonyl)-1,2,3,4-tetrahydroisoquinolines that possess low affinity toward the alpha2-adrenoceptor. *J Med Chem*. 2004;47:4483-4493


**APPENDIX B: GENETIC VARIATION OF THE BETA-2 ADRENERGIC RECEPTOR INFLUENCES THE CARDIOVASCULAR RESPONSE TO ALBUTEROL**

Paper is currently under review for publication in the journal of Applied Physiology, Nutrition, and Metabolism.

**ABSTRACT**

Beta-2 adrenergic receptors (B2ARs) found in the heart and vasculature regulate cardiovascular function. We have found that administering albuterol, a beta-2 receptor agonist, increases cardiac output (Q) and stroke volume (SV) and decreases systemic vascular resistance (SVR). Variation of the gene encoding the B2AR has been shown to influence cardiovascular function. An arginine to glycine substitution at amino acid position 16 is commonly studied. We sought to determine if the cardiovascular response to albuterol differed between the homozygous arginine condition at amino acid 16 (AA, n=6), the homozygous glycine condition (GG, n=17), and the heterozygous condition (AG, n=19). We measured cardiac output (Q), heart rate (HR), and systolic and diastolic blood pressure. Stroke volume (SV), mean arterial blood pressure (MAP), and systemic vascular resistance (SVR) were calculated. Baseline and 60 minutes post-albuterol measurements were performed. The genotype groups had similar cardiovascular parameters at baseline. At 60 minutes post-albuterol, the AG genotype exhibited a greater drop in SVR compared to AA and GG subjects. These differences in percent change of Q, SV, HR, MAP, or blood pressures between genotype groups were not statistically different; however,
some of these measures were drastically different between genotype, despite not reaching statistical significance. These results suggest that genetic variation of the B2AR perhaps plays an influential role in the cardiovascular response to albuterol.

**INTRODUCTION**

Beta-2 adrenergic receptors (B2ARs) in the heart and systemic vasculature play a pivotal role in sympathetic regulation of the cardiovascular system. Sympathetic agents epinephrine and norepinephrine both bind to B2ARs; however, epinephrine has a 10-50 fold greater affinity for the receptor\(^{47, 109}\). The main cardiovascular effects of B2ARs occur through the recruitment of the G\(_S\)-protein pathway, subsequently increasing intracellular cAMP and active PKA. These intracellular events trigger a cascade of reactions that ultimately result in vasodilation and cardiac inotropy\(^{55, 110}\). Transgenic studies show further evidence of the role of B2ARs on cardiovascular regulation. Knockout of the gene encoding the B2AR in the vasculature blunts vasodilatory responses in response to the non-selective beta agonist isoproterenol\(^{111, 112}\). Similarly, mice that overexpress the human B2AR sixty times fold display significant increases in cardiac output (Q) and stroke volume (SV)\(^{113}\).

Structurally, the B2AR has seven membrane-spanning domains with an extracellular amino-terminus and a cytoplasmic carboxyl-terminus\(^{54}\). The receptor is encoded by a single intronless and highly polymorphic gene on
chromosome 5q31-32. This receptor is associated with three commonly-studied single nucleotide polymorphisms with functional effects both in vivo and in vitro. Among the most studied is the arginine (Arg) to glycine (Gly) substitution at amino acid 16 (Arg16Gly) found in the receptor's extracellular amino-terminus. A homozygous arginine condition (AA) at amino acid 16 is associated with lower Q, SV, and mean arterial blood pressure (MAP), both at rest and during exercise, when compared to both the heterozygous (AG) and homozygous glycine (GG) conditions. Similarly, arginine homozygotes exhibit smaller increases in heart rate (HR) and Q compared to the glycine homozygote group during isometric hand grip exercise. Furthermore, AA subjects have decreased ejection fraction and fractional shortening when compared to subjects AG and GG subjects. Vascular effects of arginine homozygosity include decreased vasodilatation and desensitization in response to administration of the non-selective beta agonist isoproterenol. Overall, these studies collectively demonstrate that the AA condition is comparably unfavorable from a cardiovascular standpoint as compared to the GG individuals. This trend of blunted cardiovascular function in the arginine 16 homozygous condition could be explained by reduced receptor concentration as measured in lymphocytes.

Investigations of the B2AR polymorphism have determined phenotypic differences according to genotype by stimulating receptors via endogenous catecholamines such as those released during exercise or exogenous agonists which have been administered systemically. To our knowledge, one study, to
date, has addressed differential responses to the inhaled short-acting beta-2 agonist albuterol according to B2AR polymorphisms. However, this study focused primarily on respiratory parameters, serum potassium, and only reports two cardiovascular parameters (HR and diastolic blood pressure). Therefore, we sought to determine the effect of genetic variation of the B2AR at amino acid position 16 on the cardiovascular response to inhaled albuterol. We hypothesized that genetic variation of B2AR would lead to differential albuterol-induced cardiovascular responses (Q, HR, SV, systolic and diastolic blood pressures, and systemic vascular resistance).

METHODS:

Subjects

Forty-two subjects were recruited and stratified according to genotype (AA, n=6; AG, n=19; GG, n=17). The subjects provided informed consent prior to the start of the study. The protocol was approved by the University of Arizona Institutional Review Board and all aspects of the study were in accordance with the latest revision of the Declaration of Helsinki.

Protocol

Subjects arrived to the laboratory in a fasted state. The first visit to the laboratory was a screening visit to rule out exclusion criteria. During the screening visit, subjects underwent a maximal exercise test to exhaustion to obviate cardiovascular abnormalities, a complete blood count to detect anemia and/or kidney dysfunction, and a pregnancy test in women. Smoking was also
considered an exclusion criterion. For the maximal exercise test, workload on a cycle ergometer was incrementally increased every three minutes until exhaustion. Testing was ended when RER became greater than 1.15, the subject could no longer maintain a pedal rate of >60RPM, the subject’s rating of perceived exertion on the Borg scale reached 18 out of 20, HR was >100% predicted, or SaO\textsubscript{2} dropped below 85%\textsuperscript{120}. Oxygen uptake (VO\textsubscript{2}) and production of carbon dioxide (VCO\textsubscript{2}) were monitored using a Medical Graphics CPX/D (St. Paul, MN) metabolic cart interfaced with a Perkin Elmer MGA-1100 mass spectrometer (Perkin-Elmer 1100, Welsley, MA) as described previously\textsuperscript{117}. Heart rate was monitored during both study visits via an electrocardiogram (Marquette Electronics, Milwaukee, WI USA).

On the study visit (second visit to laboratory), subjects again arrived in a fasted state. Baseline cardiovascular measurements including assessment of cardiac output, heart rate, and blood pressure were done in triplicate at the beginning of the second visit. Following baseline measurements, albuterol (nebulized albuterol sulfate, 2.5mg diluted in 3ml of normal saline) was administered via a Power Neb2 nebulizer (Port Washington, New York). Subjects were asked to breathe quietly until all liquid had been nebulized, which took approximately 12 minutes. Subjects were instructed to breathe deeply every two minutes to ensure dispersal of nebulized particles into the lower airways. Cardiac output, heart rate, and blood pressure measurements were again taken 60 minutes post-albuterol administration.
Genotyping

Genomic DNA extracted from buccal swabs was used for genotyping of the B2AR polymorphism at amino acid 16. A custom magnetic bead protocol using SILANE chemistry (Life Technologies, Carlsbad, CA) on BioSprint96 (QIAGEN, Gaithersburg, MD) was utilized. Samples were incubated at 55°C for 4-8 hours with Proteinase K prior to extraction. DNA quantitation was performed using PicoGreen (Life Technologies) was used for quntitation of genomic DNA. All samples were normalized to a final concentration of 5 ng/µl. Pre-validated primers and probes for the TaqMan Allelic Discrimination Assay were obtained from Life Technologies (rs#1042713). Reactions were set up in 10µL on 384-well plates using TaqMan Universal PCR Master Mix, No AmpErase® UNG (Life Technologies) with 10ng DNA, and 1X Assay Mix, including one no template control (NTC) and one blank containing no mastermix. The thermal cycling reactions (95 °C for 10 minutes, 50 cycles of 92 °C for 15 seconds and 60 °C for 1 minute) were analyzed on a 7900 Real-Time PCR System (Life Technologies) including a pre-read protocol for removal of background fluorescence run at 60 °C for 1 min. All samples were analyzed with Applied Biosystems Genotyper software (SDS system, version 2.3).

Assessment of Q, HR, SV, MAP, BP, and SVR

Cardiac output (Q) was determined using the 8-10 breath open-circuit acetylene rebreathe technique as previously described. A pneumotach was connected to a non-rebreathing Y valve with the inspiratory port connected to a
pneumatic valve (Hans Rudolph, KC, MO) thus allowing rapid switching from room air to test gas mixture (5-liter rebreathe bag containing 0.7% C\textsubscript{2}H\textsubscript{2}, 21%O\textsubscript{2}, 9% He, and N\textsubscript{2}). Gas sampling was done via mass spectroscopy (Perkin-Elmer). Integration with custom analysis software allowed Q assessment. Heart rate (HR) was measured with a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI USA). Along with Q, HR was used to determine stroke volume (SV) through a formula: \(SV=Q/HR \times 1000\). A sphygmomanometer equipped with a stethoscope was used to measure systolic (SBP) and diastolic blood pressure (DBP); the auscultation technique was utilized with the same technician performing all measures. Mean arterial pressure (MAP) was calculated using \(MAP=DBP+\frac{1}{3}(SBP-DBP)\). SVR was calculated using \(SVR=\frac{(MAP-\text{Central Venous Pressure} \times 80)}{Q}\), where central venous pressure is assumed to be 5mmHg \(^8\).

**Statistical Analysis**

All statistical comparisons were performed using the SPSS statistical software package (v. 17.0, Chicago, IL). Mean values for cardiovascular measurements were reported as averages of the triplicate measures for both time points (baseline and 60 minutes post-nebulization); these were presented in the mean±SE format, unless otherwise specified. Statistical comparisons between the three genotype groups (AA, AG, GG) were done using ANOVA testing with significance set at an \(\alpha\) level of 0.05.
RESULTS:

Subjects across genotype groups were similar in height and age (Table 1). However, the AA group exhibited a higher weight and body surface area (BSA). Therefore, cardiac output (Q) and stroke volumes are reported as cardiac index (QI) and stroke volume index (SVI) in order to correct for BSA, respectively. Additionally, the groups differed in gender; the AG group had a higher percentage of females (70%) compared to the AA group (22% female) and the GG group (38% female) (Table 1).

All baseline measures (i.e. prior to albuterol administration) were similar between the three genotype groups (HR, QI, SVI, SBP, DBP, MAP, and SVR; Table 2). There were no statistically significant differences in percent change from baseline to 60 minutes post-albuterol between genotypes for QI, SVI, and HR (Figures 1, 2 and 3, respectively). Furthermore, no differences in SBP or DBP were found between genotypes (SBP: -4±4, -2±2, 0.01±2; DBP: -2±4, -3±2, -0.01±2; for AA, AG, GG, respectively). However, there was a difference in percent change for SVR between genotype groups (Figure 4). The AG genotype group exhibited a larger drop in SVR in response to albuterol administration, followed by the GG genotype group. In contrast, the AA group showed a rise in SVR in response to albuterol.
Discussion

In the present study we demonstrate that genetic variation of the beta-2 adrenergic receptor at amino acid position 16 may influence the change in systemic vascular resistance in response to albuterol. We tested the three possible genotypes of the polymorphism at amino acid position 16, GG, AG, and AA; we found that the AG genotype group exhibited a significantly larger drop in systemic vascular resistance (SVR) compared to the GG and AA groups. The AA group exhibited an increase in SVR in response to albuterol administration. SVR, or the resistance offered by the peripheral circulation, is an indirect reflection of vasodilation and vasoconstriction. The increase in catecholamines that occurs in response to albuterol bind to B2ARs in the vasculature to mediate vasodilation in the same manner as circulating epinephrine and norepinephrine. In this context, our results suggest that the B2ARs of the GG or AG genotype are perhaps more successful at mediating a vasodilatory response when agonist stimulated when compared to the AA genotype.

We found no significant difference in percent change from baseline to 60 minutes post-albuterol administration in regards to cardiac index, stroke volume index, heart rate, and blood pressures. Despite this lack of significance, we would like to point out that the differences between genotypes in regards to cardiac output, stroke volume, and heart rate are rather large. Specifically, the GG and AA groups widely differ in percent change of cardiac output and stroke volume. Furthermore, the AG and AA groups differ largely in heart rate percent change. We want to highlight these large differences and perhaps larger subject
numbers would lead to statistically significant changes. Our results clearly show genotypic differences in the cardiovascular response to albuterol and therefore beg for future studies to further explore the influence of the polymorphism of the B2AR’s amino acid 16.

Adrenergic receptors mediate the cardiovascular response to sympathetic nervous system activation through binding of catecholamines. B2ARs bind both plasma epinephrine, whose sole source is the adrenal medulla, and norepinephrine, which can derive from the adrenal medulla and post-ganglionic sympathetic fibers; however, the B2AR has a higher affinity for epinephrine. B2ARs mediate cardiac inotropy and vasorelaxation, especially within the muscular vasculature. The beta 2 adrenergic receptor is a classic G-protein coupled receptor that mediates these cardiac and vascular effects via adenylyl cyclase activation and subsequent increases in cAMP and active PKA. In the heart, PKA phosphorylates L-type Ca\(^{2+}\) channels resulting in increased calcium influx into the cytosol. Calcium flux through L-type Ca\(^{2+}\) channels triggers calcium-induced calcium release and ultimately contraction via troponin-mediated mechanisms. In the vasculature, the increase in cAMP due to B2AR stimulation acts as an inhibitor of myosin light chain kinase (MLCK). Normally, MLCK phosphorylates the myosin light chain to promote crossbridge formation between myosin and actin. Therefore, B2AR-mediated inhibition of MLCK decreases contractile force and promotes blood vessel relaxation.

The B2AR is involved in multiple cardiovascular and renal pathways that influence the control of blood pressure and the B2AR gene has been identified as
a candidate gene for hypertension. Our results show no genotypic differences in the blood pressure response to albuterol; however, previous studies support the influence of the B2AR polymorphism at amino acid 16 on blood pressure dysregulation. For example, a recent association study found that the G allele at amino acid 16 is associated with essential hypertension in a Northern Han Chinese population. Similar studies associate the G allele to hypertension in Asians but no association has been found in American whites or blacks.

The association of the G allele with hypertension, despite tendency of the G allele to confer increased vasodilation and decreased systemic vascular resistance, can be possible explained by higher sodium reabsorption of the GG genotype as previously evidenced.

Nebulized albuterol sulfate was the beta 2-agonist used in our study. Albuterol is the first-line treatment for respiratory conditions such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis. Albuterol binds to beta-2 adrenergic receptors causing bronchodilation and both fluid and mucociliary clearance. We have previously shown that albuterol increases catecholamines. Because the cardiovascular system is regulated by beta-2 adrenergic receptors, the combination of albuterol and albuterol-mediated increases in catecholamines could lead to an elevated cardiovascular state in patients with respiratory disease taking albuterol. Our results in conjunction with previous studies show that asthma or cystic fibrosis patients with glycine homozygosity at amino acid position 16 of the B2AR would display unwanted...
side-effect of elevated cardiovascular activity when compared to patients with arginine homozygozity at this same position.

Conclusions

In summary, we found that genetic variation of the B2AR at amino acid position 16 may influence the change in systemic vascular resistance in response to albuterol administration. Our results suggest that the polymorphism at position 16 does not affect cardiac output, heart rate, or blood pressure responses to albuterol but we studied a relatively small amount of subjects for each genotype group. It is also possible that other variant, including others within the B2AR gene, have participating roles in the cardiovascular response to beta-2 agonist administration. Further mechanistic studies with higher subject numbers will help further elucidate the role of B2AR polymorphisms in the physiological response to albuterol.
Table 1. Subject Characteristics

<table>
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<tr>
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<td>VO₂peak</td>
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<td>34±4</td>
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Mean ± SE. BMI=Body mass index, BSA= Body surface area, VO₂peak= peak oxygen consumption, *p<0.05 between groups.
### Table 2. Baseline Measurements

<table>
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<td>HR (beats/minute)</td>
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<td>DBP (mmHg)</td>
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<td>MAP (mmHg)</td>
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<td>SVR (dynes*sec/cm⁵)</td>
<td>966±95</td>
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</table>

Mean±SE.

Mean±SE. QI: Cardiac Output Index; SVI: Stroke Volume Index; HR: Heart Rate; SBP: Systolic Blood Pressure; MAP: Mean Arterial Blood Pressure; SVR: Systemic Vascular Resistance
Figures

Figure 1

Fig. 1 Percent change of cardiac index from baseline to 60 minutes post-albuterol administration, according to genotype group. The error bars represent the SE of the mean.
**Figure 2**

![Bar graph showing percent change of stroke volume index from baseline to 60 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.]

**Fig. 2:** Percent change of stroke volume index from baseline to 60 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.
**Fig. 3:** Percent change of heart rate from baseline to 60 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.
**Fig. 4:** Percent change of systemic vascular resistance from baseline to 60 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean. *P <0.05 between groups.
References


13. Tang W, Devereux RB, Kitzman DW, Province MA, Leppert M, Oberman A, Hopkins PN, Arnett DK. The arg16gly polymorphism of the beta2-


APPENDIX C: GENETIC VARIATION OF THE BETA-2 ADRENERGIC RECEPTOR AND THE BRONCHODILATOR RESPONSE TO ALBUTEROL IN CYSTIC FIBROSIS

Abstract

We sought to determine the influence of genetic variation of the beta-2 adrenergic receptor on the airway response to albuterol in patients with cystic fibrosis (CF) from baseline to 30-, 60-, and 90-minutes post-albuterol administration compared to healthy controls; the polymorphism results in an amino acid change in position 16 consisting of an arginine (A) to glycine (G) substitution. Baseline pulmonary function (forced vital capacity, FVC, forced expiratory volume in 1-second, FEV\textsubscript{1}, maximal mid-expiratory flow, MMF, and forced expiratory flow at 50% of the FVC, FEF\textsubscript{50}) was assessed in 17 patients with CF and 31 healthy subjects. As expected, the healthy group had higher baseline pulmonary function compared to the CF group. We compared homozygous arginine subjects (AA) to subjects with a glycine-containing genotype (AG/GG). There was no effect of genotype on the response to albuterol in healthy subjects. However, in the CF group, we found that the AA group (n=6) had an attenuated response to albuterol when compared to the glycine-containing group (n=11) (FVC=1±1 vs. 6±3; FEV\textsubscript{1}=3±1 vs. 7±4; MMF=12±4 vs. 12±5; FEF50=10±3 vs. 15±5 % change, for AA vs. AG/GG group, respectively, p<0.05 for FVC). These results demonstrate a differential response to beta-2 agonists according to genetic variation of the beta-2 adrenergic receptor at amino acid 16 in CF subjects. Due to the differences in FVC, these data suggest that
the difference in airway function exhibited in this study is primarily due to bronchodilation of the larger airways.

**Introduction**

Cystic fibrosis (CF) is the most common genetic disease in Caucasians and affects approximately 30,000 Americans\textsuperscript{132,133}. CF is a chronic condition caused by a genetic defect of the cystic fibrosis transmembrane conductance regulator (CFTR) located in the apical membrane of epithelial tissue. CFTR plays important roles in both chloride transport and the down-regulation of sodium transport through the epithelial sodium channel (ENaC)\textsuperscript{134,135}. Hyperabsorption of sodium and a decrease in chloride transport are hallmarks of CF, leading to gastrointestinal, reproductive, and lung dysfunction. In the lung, dysregulated ion transport leads to decreased airway surface fluid and increased viscosity of airway secretions. The resulting impairment in mucociliary clearance makes CF patients highly prone to bacterial infection and a constant inflammatory state\textsuperscript{136}. The ΔF508 mutation is the most common CFTR mutation in CF and results in a lack of CFTR in lung epithelium due to premature proteolysis of the mutant protein\textsuperscript{137}. This mutation is present in 90% of the CF population\textsuperscript{138}.

Inhaled bronchodilators such as albuterol constitute the treatment regimen for CF and other respiratory conditions including asthma\textsuperscript{128}. Albuterol, a beta-2 receptor agonist, enhances mucociliary clearance, decreases work of breathing, and improves lung mechanics in the CF lung\textsuperscript{130}. The widespread clinical use of albuterol in CF contrasts to the paucity of data of its effectiveness in the CF lung;
however, studies do suggest that albuterol improves $FEV_1$ in CF patients$^{129}$. Significant interindividual variability exists in the bronchodilator response to beta-2 agonists, suggesting that a genetic component may dictate how effective a therapy albuterol may be in CF patients$^{139}$.

Several polymorphisms for the beta-2 adrenergic receptor (B2AR) exist, including an arginine (Arg) to glycine (Gly) substitution at amino acid position 16 (nucleotide 46). Several studies have attempted to elucidate the possible influence of B2AR genetic variation on pulmonary function. One study demonstrates that healthy subjects homozygous for arginine at amino acid position 16 exhibit greater lung fluid accumulation compared to glycine homozygotes in response to an intravenous saline load; this suggests that glycine homozygotes have elevated B2AR activity$^{69}$. Healthy arginine and glycine homozygote adults do not seem to differ in the bronchodilatory response to exercise, as measured by forced expiratory flow at 50% of the forced vital capacity (FEF$_{50}$). However, glycine homozygotes have sustained bronchodilation during recovery from exercise, suggesting that arginine homozygosity grants enhanced desensitization of the beta-2 adrenergic receptor to endogenous catecholamines$^{140}$. A study in heart failure patients found decreased pulmonary function in ArgArg subjects but no genotypic differences in healthy subjects, as measured by decreased measures of vital capacity, forced vital capacity (FVC), forced expiratory flow in 1-second (FEV$_1$), and forced expiratory flow rate at 25-75% of the forced vital capacity (MMF); this suggests that the B2AR polymorphism at amino acid 16 has an effect on B2AR desensitization in
response to the elevated sympathetic state characteristic of heart failure. Cardiovascular studies of the B2AR also support that arginine homozygozity at amino acid position 16 lead to decreased function and increased desensitization. Despite the abundance of studies of the B2AR polymorphism at amino acid position 16, no study to date has investigated the possible influence of this polymorphism on the pulmonary response to albuterol in CF. We, therefore, sought to determine whether genetic variation of the B2AR at amino acid position 16 affects pulmonary function in CF patients following albuterol administration. We hypothesized that subjects whose genotype included a copy of glycine (AG/GG) would exhibit an elevated pulmonary response to albuterol as compared to arginine homozygotes (AA).

Methods

Subjects

Seventeen CF patients and thirty-one healthy subjects were recruited for this study. CF was confirmed with a positive sweat test and having at least one ΔF508 allele. Clinical stability, a pre-requisite for the study, was determined by implementing the following exclusion criteria: FEV₁ ≤ 40% predicted, currently taking experimental CF drugs, pulmonary exacerbation within the last six months resulting in ≥50cc of blood in the sputum, antibiotics for pulmonary exacerbation, pregnancy, smoking, inability to exercise, or cardiovascular abnormalities. Subject stratification was done according to variation at amino acid 16 of the B2AR; subjects were either part of a non-glycine containing group (AA) or a
glycine-containing group (AG/GG). The protocol was reviewed and approved by the University of Arizona Institutional Review Board and all aspects of the study were performed according to the latest revision of the Declaration of Helsinki. All participants provided written informed consent prior to study.

Protocol

Upon arrival to the laboratory in a 2-hour fasted state (nothing but water for three hours prior, including refraining from caffeine), subjects underwent buccal swabbing for genotyping of the B2AR polymorphism and for confirmation of the ∆F508 allele (CF subjects). Subjects were placed on a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI) for continuous heart rate monitoring throughout the study visit. A 20-gauge venous catheter was placed in each subject and a baseline blood sample was taken. Subjects underwent baseline pulmonary function testing maneuvers as described further below. Nebulized albuterol was administered (2.5mg diluted in 3 ml saline) using a Power Neb2 nebulizer (Drive Medical, Port Washington, NY). Subjects were instructed to sit and breathe quietly until all the albuterol was administered via a nosepiece, typically for ten to twelve minutes. Subjects were instructed to take a deep breath every two minutes to allow dispersal of the nebulized particles into the lower airways. Blood samples were taken and pulmonary function testing performed at three time points following albuterol administration (30, 60, and 90 minutes post-albuterol).
Pulmonary Function Testing

All pulmonary function tests were done according to American Thoracic Society standards. Subjects performed forced expiratory volume and flow maneuvers in a seated position at both baseline and at 30-, 60-, and 90- minutes post-albuterol administration. Forced vital capacity (FVC), forced expiratory volume after one second (FEV₁), forced expiratory flow at 50% of the FVC, (FEF₅₀) and forced expiratory flow rate at 25-75% of the forced vital capacity (also called mid-maximal expiratory flow ,MMF) were assessed from the forced expiratory volume and flow maneuvers. All subjects were coached to take a gradual but maximal inspiration followed by a forced exhalation. Percent predicted values were determined based on predicted equations as set by NHANES III ¹⁴², ¹⁴³.

Statistical Analyses

All statistical comparisons were performed using the SPSS statistical software package (v. 19.0; SPSS, Inc., Chicago, IL). Mean values for all parameters were calculated as an average and were presented in the mean ± standard error format unless otherwise stated. Main effects and interactions were determined using a repeated measures ANOVA. After confirming equality of variance with a Levene’s test, percentage comparisons between genotype groups were performed using an independent samples t-test with significance set at an α level of 0.05.
Results

Subject characteristics

Thirty-one healthy and seventeen CF subjects were recruited and completed the study (Table 1: Subject characteristics). Seven of the healthy subjects had the AA genotype and twenty-four were part of the Ag/GG genotype group. Six CF subjects had the AA genotype and 11 had at least one copy of glycine at amino acid position 16 of the B2AR (AG/GG). No significant differences existed between healthy and CF subjects in gender (% female), age, height, or body mass index (BMI). As expected, CF subjects exhibited lower weight, \( VO_{2peak} \), FVC (% predicted), FEV\(_1\) (% predicted) and FEV\(_1\)/FVC, compared to healthy subjects.

Post-albuterol pulmonary function in healthy subjects

There was no change in FVC, FEV\(_1\), MMF, and FEF\(_{50}\) from baseline to 90 minutes post-albuterol administration in both genotypes among healthy subjects (\( F=.035, p=.965; F=.799, p=.455; F=.884, p=.419; F=.675, p=.513 \); main effect of time following albuterol administration for FVC, FEV\(_1\), MMF, and FEF\(_{50}\) respectively). Furthermore, within the healthy subjects, the response of FVC, FEV\(_1\), MMF, and FEF\(_{50}\) over 90 minutes post-albuterol did not differ between genotypes (\( F=.899, p=.351; F=.877, p=.357; F=.011, p=.917; F=.012, p=.913 \); main effect of genotype on FVC, FEV\(_1\), MMF, and FEF\(_{50}\) respectively). Also, no interaction was found between FVC, FEV\(_1\), MMF, and FEF\(_{50}\), individually, with B2AR genotype (\( F=.177, p=.975; F=.123, p=.885; F=.041, p=.96; F=.227, \)
p=.566; FVC, FEV$_1$, MMF, and FEF$_{50}$ respectively). No significant differences between genotypes in pulmonary function percent change at any timepoint (30-, 60, nor 90-minutes post-albuterol; Tables 2, 3, and 4, respectively).

**Post-albuterol pulmonary function in CF patients**

There was no change in FVC, FEV$_1$, MMF, and FEF$_{50}$ from baseline to 90 minutes post-albuterol administration in both genotypes among CF patients (F=.703, p=.503; F=.382, p=.620; F=.296, p=.665; F=.335, p=.639; main effect of FVC, FEV$_1$, MMF, and FEF$_{50}$ respectively). Furthermore, the response of FVC, FEV$_1$, MMF, and FEF$_{50}$ over 90 minutes post-albuterol did not differ between genotypes (F= .887, p=.361; F=1.74, p=.21; F=2.3, p=.15; F=1.9, p=1.8; main effect of genotype on FVC, FEV$_1$, MMF, and FEF$_{50}$ respectively). Lastly, no interaction exists between FVC, FEV$_1$, MMF, and FEF$_{50}$, individually, with B2AR genotype (F=.792, p=.472; F=.406, p=.605; F=.473, p=.56; F=.404, p=.597; FVC, FEV$_1$, MMF, and FEF$_{50}$ respectively). However, we found that percent change of FVC from baseline to 30 minutes post-albuterol administration differs between genotypes. CF subjects with glycine-containing genotypes showed a larger percent change in FVC 30 minutes following albuterol administration as compared to arginine homozygotes (Figure 1). Percent changes of FEV$_1$, MMF, and FEF$_{50}$ from baseline to 30 minutes post-albuterol administration were similar between genotypes (FEV$_1$=1±3 vs. 8±3 % change (Figure 2); MMF=4±7 vs. 12±4 % change (Figure 3); FEF$_{50}$=3±10 vs. 15±4% (Figure 4); percent change for AA vs. AG/GG groups, respectively). Percent changes for all respiratory measures from baseline to 60 minutes were similar between genotypes (FVC=-1±1 vs. 6±3
FEV$_1$=3±1 vs. 7±4 % change; MMF=12±4 vs. 12±5 % change; FEF50=10±3 vs. 15±5% change; percent change for Arg16Arg vs. Gly-containing groups, respectively) (Table 5). Similar results were obtained for the 90 minute time point (FVC=0.78±1 vs. 6.1±3 FEV1=2.6±1 vs. 7.9±4 % change; MMF=8.9±3 vs. 13.3±6 % change; FEF50=5.9±2 vs. 15.7±7% change; percent change for Arg16Arg vs. Gly-containing groups, respectively) (Table 6).

Discussion

In the present study we demonstrate that genetic variation of the B2AR influences the pulmonary response at 30 minutes post-albuterol administration in CF. Specifically, CF subjects with glycine-containing genotypes had a higher percent change in FVC compared to those with the non-glycine containing genotype. Percent change of FEV$_1$, MMF, or FEF$_{50}$ in response to albuterol did not differ between genotypes within the CF subjects at 30-minutes post-albuterol; no genotypic differences at 60- or 90-minutes post-albuterol for CF subjects. In contrast, we found no difference between genotypes of the B2AR in healthy adults at any timepoint. Overall, our results suggest that glycine containing genotypes of B2AR’s amino acid position 16 lead to enhanced pulmonary responsiveness in response to albuterol at 30 minutes after administration in CF subjects. These results suggest that a subset of CF patients would most benefit from beta-2 agonist therapy.

Beta 2 adrenergic stimulation, through agonists such as albuterol, makes up an important facet of CF treatment. Albuterol, a short-acting beta-2 agonist,
has a 15-minute onset of action and a duration of action of 5-8 hours. It helps improve work of breathing in CF patients and its mechanism of action is through the B2AR. Like all G-protein coupled receptors, the B2AR has seven transmembrane domains, and extracellular amino terminus, and an intracellular carboxyl terminus. Albuterol binds and leads to cAMP-mediated PKA stimulation. In turn, phosphorylation by PKA relaxes pulmonary smooth muscle causing bronchodilation. In contrast, albuterol binds to B2ARs in alveoli to aid lung fluid clearance. The benefit of beta-2 adrenergic stimulation in the CF lung has led to the idea of exercise, a state of elevated endogenous beta-2 agonist, as an appropriate therapy for CF. However, exercise has not been adopted as a concrete therapy in the clinical setting and reliance on exogenous beta-2 agonists persists; in fact, over 80% of cystic fibrosis patients use beta-2 agonists for pulmonary improvement. Therefore, studies on the pharmacogenetics of the beta-2 adrenergic receptor help to elucidate the basis for efficiency of albuterol and other adrenergic agonists.

Conclusions

In conclusion, we found that genetic variation of the B2AR at amino acid position 16 can influence the pulmonary response to albuterol in CF patients at 30 minutes post-albuterol administration. The polymorphism does not seem to affect the response to albuterol in healthy adults. The previously recorded variability in the pulmonary response to beta agonists in CF could be possibly explained by genetic variation of the adrenergic stimulation pathway, as suggested by our present study. Our study suggests that having the glycine
amino acid at position 16 of the B2AR leads a more active receptor response. Studies of the B2AR polymorphism at amino acid 16 and its role in the cardiovascular system also support that glycine at position 16 lead to enhanced form of the receptor. Enhanced desensitization seen in arginine homozygotes could provide an explanation as to decreases responsiveness to albuterol in CF subjects with this genotype, especially considering regular use of albuterol in CF. Furthermore, studies have suggested that glycine homozygotes have increased B2AR numbers, providing another possible reason for the difference in pulmonary function between genotypes.
Tables

Table 1: Subject Characteristics

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<td>$\text{VO}_{2\text{peak}}$ (% predicted)</td>
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<td>FVC (% predicted)</td>
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<td>FEV$_1$ (% predicted)</td>
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<td>FEV$_1$/FVC</td>
<td>0.82±0.08</td>
<td>0.71±0.12*</td>
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Values are mean±SD; BMI=body mass index; FVC=forced vital capacity; FEV$_1$=forced expiratory flow volume in 1 second of FVC; *p<0.05

Table 2: Percent Change in Pulmonary Function from Baseline to 30mins Post-Albuterol: Healthy

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<td>FVC</td>
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<td>MMF</td>
<td>23.4±4</td>
<td>19.1±4</td>
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<tr>
<td>FEF$_{50}$</td>
<td>31.5±8</td>
<td>18.3±4</td>
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Mean±SE. FVC: Forced Vital Capacity; FEV$_1$: Forced Expiratory Volume in 1 second of FVC; MMF: Forced Expiratory Flow Rate at 25-75% of the FVC; FEF50: Forced Expiratory Flow at 50% of FVC.
Table 3: Percent Change in Pulmonary Function from Baseline to 60mins

Post-Albuterol: Healthy

<table>
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<tr>
<td>FEV₁</td>
<td>5.8±1</td>
<td>2.7±2</td>
</tr>
<tr>
<td>MMF</td>
<td>20±3</td>
<td>17.6±4</td>
</tr>
<tr>
<td>FEF₅₀</td>
<td>27.4±8</td>
<td>17.4±4</td>
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Mean±SE. FVC: Forced Vital Capacity; FEV₁: Forced Expiratory Volume in 1 second of FVC; MMF: Forced Expiratory Flow Rate at 25-75% of the FVC; FEF₅₀: Forced Expiratory Flow at 50% of FVC.

Table 4: Percent Change in Pulmonary Function from Baseline to 90mins

Post-Albuterol: Healthy

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<tr>
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<tr>
<td>FEV₁</td>
<td>6.5±1</td>
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<tr>
<td>MMF</td>
<td>21.3±4</td>
<td>16.5±4</td>
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<tr>
<td>FEF₅₀</td>
<td>29.8±8</td>
<td>15.3±4</td>
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Mean±SE. FVC: Forced Vital Capacity; FEV₁: Forced Expiratory Volume in 1 second of FVC; MMF: Forced Expiratory Flow Rate at 25-75% of the FVC; FEF₅₀: Forced Expiratory Flow at 50% of FVC.
Table 5: Percent Change in Pulmonary Function from Baseline to 60mins Post-Albuterol: CF

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<tr>
<td>MMF</td>
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<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt;</td>
<td>10.1±3</td>
<td>14.8±5</td>
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Mean±SE. FVC: Forced Vital Capacity; FEV<sub>1</sub>: Forced Expiratory Volume in 1 second of FVC; MMF: Forced Expiratory Flow Rate at 25-75% of the FVC; FEF<sub>50</sub>: Forced Expiratory Flow at 50% of FVC.

Table 6: Percent Change in Pulmonary Function from Baseline to 90mins Post-Albuterol: CF

<table>
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<tr>
<th></th>
<th>AA</th>
<th>AG/GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>0.78±1</td>
<td>6.1±3</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2.6±1</td>
<td>7.9±4</td>
</tr>
<tr>
<td>MMF</td>
<td>8.9±3</td>
<td>13.3±6</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt;</td>
<td>5.9±2</td>
<td>15.7±7</td>
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</tbody>
</table>

Mean±SE. FVC: Forced Vital Capacity; FEV<sub>1</sub>: Forced Expiratory Volume in 1 second of FVC; MMF: Forced Expiratory Flow Rate at 25-75% of the FVC; FEF<sub>50</sub>: Forced Expiratory Flow at 50% of FVC.
Figure 1: Percent change of forced vital capacity (FVC) from baseline to 30 minutes post-albuterol in CF subjects, according to genotype group. The error bars represent the SE of the mean. *P <0.05 between groups.
Figure 2: Percent change of forced expiratory volume in 1 second of FVC (FEV₁) from baseline to 30 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.
Figure 3: Percent change of forced expiratory flow rate at 25-75% of FVC (MMF) from baseline to 30 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.
Figure 4: Percent change of forced expiratory flow at 50% of FVC (FEF$_{50}$) from baseline to 30 minutes post-albuterol, according to genotype group. The error bars represent the SE of the mean.
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