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Genetics of β_2 -Adrenergic Receptors and the Cardiopulmonary Response to Exercise

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Abstract

Exercise elicits a number of physiologic responses to increase oxygen delivery to working muscles. The β_2 -adrenergic receptors (ADRB2) play a role in the cardiopulmonary response to exercise. This review is focused on how the gene that encodes the ADRB2 influences the cardiopulmonary response to exercise. In addition, we discuss possible interactions between ADRB2 and other genes important in exercise performance.

Keywords

ADRB2; ADRB1; cardiac output; stroke volume; pulmonary; airway

INTRODUCTION

Exercise results in several key cardiopulmonary responses with the general outcome of improving blood — and therefore oxygen and nutrient — transport to working muscles. The cardiopulmonary response to exercise is also important in moving carbon dioxide and other metabolites away from working muscles. This exercise response uses both sympathetic and parasympathetic pathways. Catecholamines are increased minimally with light exercise but increase markedly as exercise intensity increases to 50% maximal exertion and above. With exercise, minute ventilation (VE) increases in response to the elevated demand for oxygen through augmentation of both tidal volume and respiratory rate. The initial increase in VE with light exercise is due primarily to vagal withdrawal that results in bronchodilation. With heavy exercise (and the resultant increase in circulating catecholamines), further bronchodilation is likely the result of stimulation of the β_2 -adrenergic receptors (ADRB2) from epinephrine.

Cardiac output is also augmented with light and heavy exercise through increases in both heart rate and stroke volume. As with the pulmonary response to exercise, vagal withdrawal plays a key role in the increase in heart rate and cardiac output with light exercise. As exercise intensity increases, elevations in heart rate and stroke volume are both influenced by circulating norepinephrine and epinephrine that stimulate the β_1 -adrenergic receptors (ADRB1) and ADRB2. In addition to increased alveolar ventilation and cardiac output, the regulation of blood flow distribution during exercise is important, and flow is shunted away from areas with diminished need for oxygen (such as the digestive system) to areas with elevated need (such

as the working skeletal and respiratory muscles). These alterations in blood flow are also regulated, at least in part, by circulating levels of catecholamines and the ADRB2.

The ADRB2 are found in airway and vascular smooth muscle, the atria and ventricles, and (to a lesser extent) the sinoatrial node. The focus of this review is on how common sequence variation in the gene that encodes the ADRB2 influences the cardiovascular and pulmonary response to exercise. In addition, we examine how variation in the gene that encodes the ADRB2 and genes that encode other proteins that are important in the cardiopulmonary response to exercise may interact and further influence cardiovascular and pulmonary regulation during exercise. We hypothesize that future work exploring gene-by-gene interactions between the ADRB2 and other proteins important in exercise will demonstrate a greater influence on the cardiovascular and pulmonary response to exercise, when compared with simply exploring the monogenic effects of the ADRB2 on these responses (even when more genetic information within the ADRB2, *i.e.*, haplotypes, is considered).

THE β_2 -ADRENERGIC RECEPTORS

Circulating catecholamines, norepinephrine and epinephrine, bind to the adrenergic receptors resulting in a variety of responses throughout the body (Table 1). Of the response to circulating catecholamines, the ADRB2 are primarily responsible for increases in bronchodilation, ventricular function, and vasodilation. The ADRB2 are ubiquitous seven-membrane spanning G protein-coupled receptors that primarily bind to endogenous epinephrine. Classically, stimulation of the ADRB2 leads to coupling with the stimulatory guanine nucleotide-binding protein (Gs) to activate adenylyl cyclase and increases the amount of intracellular cyclic adenosine monophosphate (cAMP). This subsequently leads to activation of protein kinase A, which phosphorylates several sarcolemmal proteins, including L-type Ca^{2+} channels and phospholamban.

The ADRB1 subtype is often classified as the primary cardiac β -receptor because stimulation of this receptor increases both heart rate and myocardial contractility. The ADRB2 is most associated with bronchiole smooth muscle and the peripheral vasculature; however, the ADRB2 are also expressed in the human heart and have been shown to influence heart rate and cardiac contractility. The ADRB2 also play a role in lipolysis, with the stimulation of these receptors increasing lipolysis.

The importance of the ADRB2 in the response to exercise has been elucidated primarily from studies using β -blockade or, more recently, using knockout models. β -Blockers, both specific and nonspecific for the ADRB1, have been shown to markedly influence bronchodilation in response to short-term exercise as well as heart rate, blood pressure, and stroke volume, therefore reducing the important cardiopulmonary augmentations that occur in response to exercise. In addition, use of β -blockers reduces exercise performance with longer duration exercise, causing the typically observed drift in oxygen uptake with more prolonged exercise (which uses a greater proportion of fat as a fuel source) to be blunted, possibly because of the effect the ADRB2 plays on lipolysis (8).

Similar to other adrenergic receptor subtypes, the ADRB2 are prone to desensitization with prolonged stimulation. This desensitization acts as an important protective mechanism to prevent undue stress from overactivity of the receptor and possible organ damage. Because the ADRB2 can still bind to an agonist without adenylyl cyclase activity, one method of desensitization likely involves phosphorylation of specific residues in the receptor protein. After phosphorylation, the receptors may be reactivated by phosphatases or internalized by endocytosis and then degraded and are eventually replaced by newly synthesized receptors.

With prolonged stimulation, it is known that there is an eventual loss of ADRB2, as measured by ligand-binding studies, which is a slower process than the uncoupling from adenylyl cyclase. Studies show that exposure to a low concentration of isoproterenol, a nonselective β -adrenergic agonist, for 8 h leads to a 10% decrease in receptor number and that recovery of this desensitization takes several days (10). The initial studies that explored ADRB2 function according to variation in the gene that encodes this receptor determined that differences in desensitization, rather than differences in the baseline number of receptors, likely contribute to alterations in the phenotypic response to an agonist. More recent work in humans, however, suggests that this is not the only factor resulting in genotype-related differences in receptor function.

GENETIC VARIATION OF THE ADRB2

There are several common single nucleotide polymorphisms (SNP) of the gene that encodes the ADRB2. In addition, there are a number of haplotype combinations based on genetic variation at multiple sites within this gene that have been described and studied (Table 2). Recently, a large (5.3 kb) portion of the ADRB2 gene has been resequenced, including an area of the 3' untranslated region (14). Of the common SNP and haplotype combinations previously studied, the most thoroughly explored genetic variants of the ADRB2 include an arginine (Arg)-to-glycine (Gly) substitution at nucleotide 46 (amino acid 16) and a glutamine-to-glutamic acid substitution at nucleotide 79 (amino acid 27). Initial *in vitro* studies demonstrated that cultured Chinese hamster fibroblast cells with the Arg variant at amino acid 16 (Arg16) had greater resistance to agonist-mediated desensitization when compared with those cells with the Gly variant at this amino acid (Gly16). Other studies in this same cell line also demonstrated that cells with the glutamine variant at amino acid 27 (Gln27) were resistant to agonist-mediated desensitization when compared with cells with the glutamic acid variant (Glu27).

Although the phenotypic response to stimulation by an agonist according to genetic variation at amino acids 16 and 27 has been studied in great detail (because they are both common and functional), the genetic variant of the ADRB2 that seems to have the greatest functional effect is a threonine-to-isoleucine substitution at codon 164 (Thr164Ile) (4). The heterozygous condition at codon 164 is rare, however, occurring in less than 5% of the white population. The Thr164Ile condition has been shown to dramatically alter desensitization in response to stimulation from an agonist in cell cultures, as well as vasodilation, left ventricular function, and survival rates in patients with heart failure (4). There is less of a consensus, however, about the functional consequence of genetic variation of the ADRB2 at amino acids 16 and 27, although a clear picture seems to be emerging in humans (Table 3).

VARIATION OF THE ADRB2 GENE AND THE CARDIOVASCULAR RESPONSE TO EXERCISE

Both the ADRB1 and the ADRB2 are important in cardiovascular regulation at rest and during exercise. There is considerable heterogeneity in the tissue distribution and function of the β -adrenergic receptor subtypes in the heart among different species. In human left ventricles, the ratio of ADRB1 to ADRB2 is 80/20, whereas in the atria, the ratio decreases to 70/30. Phosphorylation of L-type Ca^{2+} channels via ADRB2 stimulation promotes Ca^{2+} influx and, thus, enhances ventricular contraction. Also, phosphorylation of phospholamban may be involved in enhanced diastolic relaxation by increasing Ca^{2+} uptake into the sarcoplasmic reticulum. In addition to activating pathways that regulate cardiac function, ADRB2 stimulation can activate pathways that regulate cardiac growth and remodeling, and therefore, polymorphisms of these receptors could possibly alter baseline cardiovascular structure and function.

In mice, overexpression of the ADRB2 in the heart leads to increases in contractility of the myocardial tissue and cardiac output. Knockout of the ADRB2 leads to an increase in heart rate and blood pressure during exercise, whereas knockout of the ADRB1 and ADRB2 leads to decreases in stroke volume compared with wild-type mice (1). The ADRB2 is also the primary adrenergic receptor that causes vasodilation in humans. Genetic variants of the ADRB2 that alter receptor function could therefore influence the cardiovascular response to exercise through differences in cardiac output, flow of blood to working muscles, or both.

We have previously shown that subjects homozygous for Gly at amino acid 16 have greater stroke volume and cardiac output when compared with subjects homozygous for Arg at this amino acid (24). Interestingly, we and others have demonstrated a difference in cardiac function at rest and during exercise. Tang *et al.* have shown that Gly16 subjects have augmented left ventricular function (demonstrated through measurements of ejection fraction, fractional shortening, and midwall shortening) at rest when compared with Arg16 subjects, suggesting that differences in baseline receptor function (or number) rather than differences in desensitization of these receptors may be driving the phenotypic alterations as they relate to the cardiac tissue (13).

Because of the likelihood that a genotype difference in receptor density, rather than differences in agonist-mediated desensitization, may be influencing cardiac function, we have examined the density of ADRB2 on lymphocytes in healthy subjects according to sequence variation at amino acids 16 and 27 and studied if there is a relationship between ADRB2 density on lymphocytes and cardiac function (27). We assessed the density of ADRB2 on mononuclear cells because previous work using positron emission tomography and simultaneous radioligand binding analysis with mononuclear cells has demonstrated that the number of β -adrenergic receptors on lymphocytes is related to the number of β -adrenergic receptors on the myocardial tissue. We found that subjects homozygous for Gly at amino acid 16 had more ADRB2 for a given number of lymphocytes compared with subjects homozygous for Arg at this amino acid. Perhaps more importantly, we demonstrated a relationship between the amount of ADRB2 on lymphocytes and stroke volume in these subjects. Other authors, however, have not found a difference in receptor density according to genotype of the ADRB2. For example, Bruck *et al.* (3) found no differences in baseline ADRB2 density or isoproterenol-stimulated cAMP production on human lymphocytes. However, this study had a smaller sample size and combined Arg16Gly and Gly16Gly genotypes into one group when determining ADRB2 density and cAMP production. The authors in this study did conclude, however, that isoproterenol exposure resulted in a delayed down-regulation in subjects with the Glu27 genotype.

How this difference in baseline receptor function influences the cardiovascular response to more prolonged exercise has yet to be determined. Previous studies suggest an increase in ADRB2 density with an exercise duration of up to 2 h but that ADRB2 density falls below baseline an hour after exercise (11). Another study found that lymphocyte ADRB2 density and affinity were unchanged in sedentary individuals after prolonged exercise; however, the amount of isoproterenol required to elevate heart rate after exercise increased relative to that required before exercise, suggesting receptor desensitization (9). The effect of variation in the gene that encodes the ADRB2 on changes in receptor density with exercise has yet to be determined.

The influence of genetic variation of the ADRB2 on cardiovascular function persists from rest into light and heavy exercise when minimal and marked increases in catecholamines occur, respectively. We and others have found that the Arg16 group has lower cardiac output and stroke volume compared with the Gly16 group at rest, during exercise, and during recovery (6,7). Of interest, these findings are present, although different exercise modalities were used.

For instance, Eisenach *et al.* (6) used isometric handgrip as an exercise stimulus when exploring genetic variants of the ADRB2, whereas we have used cycle ergometry (24,25).

In addition to differences in cardiac function (cardiac output and stroke volume), our work and studies by others have demonstrated that genetic variation of the ADRB2 influences vascular function in response to an endogenous or exogenous agonist. We have demonstrated that haplotype combinations according to sequence variation at amino acids 16 and 27 influence systemic vascular resistance with exercise. Eisenach *et al.* (6) have demonstrated a lower systemic vascular resistance in Gly16 subjects, compared with Arg16 subjects, with isometric handgrip exercise (24,25).

The influence of the ADRB2 gene on the vascular and arterial pressor response with isoproterenol infusion and handgrip exercise has also been examined more directly (rather than inferring differences in vascular function through a calculated parameter, systemic vascular resistance) (5,12). Dishy *et al.* (5) demonstrated that the Arg16 condition is associated with a greater agonist-promoted desensitization in the venous circulation when compared with the Gly16 and that the Glu27 subjects demonstrated enhanced ADRB2 responsiveness. Garovic *et al.* (12) found that Arg16 subjects tend to show attenuated blood flow during infusion of a β -agonist in the brachial artery when compared with Gly16 subjects.

Based on these findings, future work is needed to determine the effects of prolonged endogenous agonist exposure (*i.e.*, longer-term exercise) on ADRB2 density and affinity (using mononuclear cells) according to variation in the gene that encodes the receptor and how this response influences cardiovascular function. Taken collectively, however, previous work in healthy humans demonstrates that the Gly16Glu27 haplotype of the ADRB2 likely represents a genotype that should have a “more favorable” cardiovascular response to short-term exercise. This is evident because the Gly16Glu27 haplotype is one that has demonstrated greater receptor numbers (Gly16), resistance to desensitization (Glu27), and augmented stroke volume and cardiac output at rest and during exercise (Gly16Glu27).

VARIATION OF THE ADRB2 GENE AND THE PULMONARY RESPONSE TO EXERCISE

The ADRB2 are located throughout the lungs from the trachea to the alveoli, have been localized to types I and II alveolar cells, and are important in bronchial smooth muscle relaxation and lung fluid clearance. Previous studies have suggested that changes in airway tone with exercise may occur mainly because of vagal withdrawal, whereas others suggest that this is primarily catecholamine mediated through the ADRB2. With heavy exercise, the ADRB2 likely plays a prominent role in airway smooth muscle relaxation and, hence, bronchodilation and improved alveolar ventilation. In contrast to our previous work that has demonstrated differences in cardiovascular function at rest, during exercise, and into recovery, we have only demonstrated genotype-dependent differences in airway function after heavy exercise in healthy subjects (25). Our group has not observed differences in airway function at rest or during short-term exercise in healthy subjects.

We have demonstrated, however, that subjects with the Gly16 genotype demonstrate sustained bronchodilation (determined by assessing expiratory flow rates) after heavy exercise when compared with subjects with the Arg16 genotype (who demonstrate a mild bronchoconstriction). The time course for the differences in cardiac and airway function according to genotype of the ADRB2 may be due to a greater ADRB2 reserve in the airways as compared with the cardiac tissue. Receptor reserve represents the amount of excess receptors that are available for stimulation and second messenger signaling as neighboring receptors become desensitized. Previous work indicates that there is a receptor density of approximately

85 fmol·mg⁻¹ ADRB2 in the human heart and 182 fmol·mg⁻¹ in the pulmonary tissue (18). Therefore, small differences in receptor number or function as a result of variation in the gene that encodes the ADRB2 could play a greater role on cardiac function compared with airway function because of less receptor reserve. This difference in receptor reserve would result in a greater time of agonist stimulation needed to unmask genotype-related differences in airway function compared with cardiac tissue. Interestingly, we have previously demonstrated that there are genotype-dependent differences in resting pulmonary function in patients with heart failure (a clinical disorder characterized by augmented adrenergic drive) but not in age and sex-matched healthy control subjects (28). This could support the hypothesis that the airways have greater receptor reserve, but with long term (years) of exposure to an elevated adrenergic drive, the genotype-related difference in airway function becomes unmasked.

Of interest, we have also demonstrated that Arg16 subjects have greater lung fluid accumulation when compared with Gly16 subjects after rapid saline infusion (26). In addition, heart failure patients with the Arg16 genotype have a lower diffusing capacity of the lungs for carbon monoxide (DLCO) compared with heart failure patients with the Gly16 genotype (28). The lower DLCO in the Arg16 genotype may be related to a decrease in alveolar-capillary membrane conductance in this group. One current school of thought in the pulmonary response to exercise is that the alveolar-to-arterial oxygen difference widens with progressive high-intensity exercise because of challenges in the ability of the lungs to regulate water. Stimulation of the ADRB2 on types I and II alveolar cells results in an increase in the activity and/or number of epithelial Sodium channels on the apical membrane of these cells. This increase in ENaC activity augments sodium and, therefore, water flux from the alveolar airspace into the cell. This fluid transport is completed on the basolateral portion of these cells via the Na⁺K⁺ transporter. In addition, the ADRB2 have been localized to the pulmonary lymphatics, and stimulation of these receptors on the lymphatics can result in lung fluid clearance. These findings suggest that the Arg16 genotype may represent a condition with a lesser ability to clear fluid from the lungs compared with the Gly16 genotype, which may be important in the pulmonary response to prolonged high-intensity exercise.

These findings suggest that young healthy subjects may have a large enough ADRB2 reserve in the airways to overwhelm any differences in ADRB2 function or density that occur with genetic variation of the ADRB2 at amino acids 16 and 27. A future study of interest would be to examine the influence of variation at codon 164 of the ADRB2 gene and the pulmonary response to exercise (given the dramatic differences in desensitization and cardiac function that have previously been demonstrated with the heterozygous condition of this polymorphism). Although difficult, one could speculate that more prolonged exercise may lead to a widening of differences in airway function according to genotype of the ADRB2 and that these differences may contribute to exercise performance. Taking the present evidence into account, the influence of the ADRB2 genotype does not seem to be strong enough to alter the pulmonary response to exercise but may influence pulmonary function in certain disease states, such as heart failure, where there is an elevated adrenergic drive.

INTERACTIONS BETWEEN ADRB2, OTHER GENES, AND THE CARDIOPULMONARY RESPONSE TO EXERCISE

Although several variants of the ADRB2 gene have demonstrated functional effects with respect to the cardio-pulmonary response to exercise, there is a growing interest in haplotype combinations according to multiple sites of the gene that encodes this receptor. Whereas study of variation according to haplotypes of the ADRB2 gene is gaining interest among some researchers, the primary focus remains on amino acids 16 and 27. This is likely for two primary reasons: first, much of the information of the ADRB2 gene is included by examining amino acids 16 and 27 because of linkage disequilibrium between these and other sites, and second,

even studies that have gone through the painstaking process of describing haplotype pairs according to multiple sites (of those that have garnered large enough groups for a meaningful statistical analysis) have demonstrated that the primary difference in phenotype seems to be weighted by amino acids 16 and 27; however, further study is needed in larger groups to determine whether variation at multiple sites of the ADRB2 leads to a phenotype that has a dramatic influence on the cardiopulmonary response to exercise.

What seems more interesting, and possibly more worthy of further exploration, is how gene-by-gene interactions between the gene that encodes the ADRB2 and genes that encode other proteins that are important in cardiovascular and pulmonary function affect the cardiopulmonary response to exercise. These may include other adrenergic receptors or proteins involved in norepinephrine and epinephrine synthesis and/or degradation (such as phenylethanolamine N-methyltransferase (PNMT)). These are genes of interest because the adrenergic receptors bind to epinephrine and/or norepinephrine, both of which are increased a great deal with intense exercise, and result in profoundly different responses. For example, the ADRB2 binds to epinephrine, resulting in vasodilation and an increase in blood flow to working muscles with exercise. In contrast, this same circulating epinephrine also binds to the α -adrenergic receptors, resulting in vasoconstriction and shunting of blood to areas with increased demand.

INTERACTION BETWEEN ADRB2 AND ADRB1 AND THE CARDIOPULMONARY RESPONSE TO EXERCISE

Common functional polymorphisms of the ADRB1 have been described and include variants at nucleotides 49 and 389. *In vivo* studies on the effects of dobutamine infusion (an ADRB1 agonist) in volunteers homozygous for Gly or Arg at nucleotide 389 of the ADRB1 found no difference in the increase in heart rate between genotype groups but a consistently greater increase in cardiac contractility in homozygous Arg389 subjects when compared with the Gly389 subjects (16,17). Liu *et al.* (19) studied exercise-induced increases in heart rate and systolic blood pressure in healthy Chinese male subjects homozygous for Arg389 ($n = 8$) and Gly389 ($n = 8$) before and after treatment with metoprolol (a selective ADRB1 antagonist) and found that dose-dependent decreases in resting heart rate and blood pressure were higher in the homozygous Arg389 subjects than in the homozygous Gly389 subjects after β -blockade. Similarly, the drop in maximal heart rate was greater in the Arg389 subjects with β -blockade. Limited studies in smaller numbers of subjects examining acute exercise effects on heart rate have not found an effect of variation at codon 389 of the ADRB1 on cardiovascular function, but future work in larger groups is certainly warranted based on the importance of this receptor in cardiac function and the demonstrated response to ADRB1 stimulation and blockade.

Polymorphisms at codon 49 of the ADRB1 have suggested variable effects on resting hemodynamics. In subjects of Chinese and Japanese descent, those homozygous for the Ser49 allele of the ADRB1 demonstrate higher resting heart rates than those carrying the Gly49 allele. Other studies have found an association between polymorphisms of the ADRB1 and mortality in patients with heart failure and an effect on peak $\dot{V}_2$ in the heart failure population (lower in subjects with Gly389 compared with subjects with Arg389) (30). In heart failure, patients with the Ser49/Gly389 haplotype have the lowest peak $\dot{V}_2$, whereas those with the Gly49/Arg389 haplotype demonstrate the highest peak $\dot{V}_2$ values.

Because of the functional importance of the ADRB1 and ADRB2, one can hypothesize that there may be clear gene-by-gene interactions between common functional variants of these receptors when they are considered together. For instance, it is likely that genotypes of the ADRB1 and ADRB2 that result in the most functional receptor number and/or activity, when considered together, will have a synergistic effect on the augmented cardiac response (cardiac

output mediated by both heart rate and stroke volume) when compared with genes that have demonstrated less functional receptors (Table 4). To date, no studies have examined the possible synergistic effects of variation in the genes that encode the ADRB1 and ADRB2.

INTERACTION BETWEEN ADRB2 AND THE α_1 ADRENERGIC RECEPTOR GENE AND CARDIOPULMONARY FUNCTION

The α -adrenergic receptor is the primary adrenergic receptor that causes vasoconstriction with stimulation from an agonist. The α_1 - (ADRA1) and α_2 -adrenergic receptors (ADRA2) each have three primary isoforms (A, B, and C). The most common subtype of α -adrenergic receptor in the heart and the vasculature is the ADRA1. The ADRA1 regulates the growth of cardiac myocytes and the strength of cardiac contractility. An Arg-to-cystine substitution has been demonstrated at position 492 of the ADRA1C, but this has not been shown to have a dramatic functional effect in humans. There is a common polymorphism of the ADRA2B gene that consists of an insertion (I) or deletion (D) section, and the D polymorphism has been shown to influence agonist-induced desensitization. In addition, the I/D polymorphism of the ADRA2B gene has been associated with differences in metabolic rate and sudden cardiac death; however, few studies have examined the influence of this polymorphism on the cardiovascular response to exercise in healthy humans (23).

Because of the functional importance of the α - and β -adrenergic receptors, one can hypothesize that there may also be clear gene-by-gene interactions between common functional variants of these receptors when they are considered together on the cardiovascular response to exercise. Interestingly, one study has suggested a possible interaction of the Arg389Gly polymorphisms of the ADRB1 and a polymorphism of the ADRA2C (the α_2C Del322Y325 polymorphism) and risk of heart failure in African Americans; further study of the gene-by-gene interactions between the adrenergic receptor subtypes and the cardiovascular and pulmonary response to exercise is difficult but certainly warranted (22).

INTERACTION BETWEEN ADRB2 AND THE GENE THAT ENCODES PNMT AND CARDIOPULMONARY FUNCTION

Other genes, besides those that encode for the adrenergic receptors, may also be of interest in exploring the impact of genetics on the cardiovascular and pulmonary response to exercise. Epinephrine, the primary agonist for the ADRA2 and ADRB2, is a sympathomimetic that is derived from the amino acids phenylalanine and tyrosine. The final rate-limiting step of epinephrine biosynthesis, the conversion from norepinephrine, relies on PNMT with S-adenosyl-L-methionine as the methyl donor (21). PNMT is found in adrenal medullary chromaffin cells, the neurons of the medulla oblongata, the hypothalamus, and the vagus nerve. In an important study, Manger *et al.* (20) used a technique to reduce PNMT activity in rats and found that these rats had lower epinephrine levels and blood pressure, despite high norepinephrine levels, highlighting the importance of PNMT in epinephrine synthesis and blood pressure regulation.

Although there have been few detailed studies on variation of the gene that encodes PNMT and epinephrine synthesis, several associations have been performed. Specifically, variation of the gene that encodes PNMT has been associated with multiple sclerosis, early-onset Alzheimer disease, weight loss, and essential hypertension in African Americans. In addition, the gene that encodes PNMT was recently resequenced in its entirety, and detailed functional studies were performed demonstrating several additional polymorphisms and a clear functional effect of variation in this gene on epinephrine synthesis, *in vitro* (15). Because of the known functional effects at amino acid 16 of the ADRB2 and the functional effects of the gene that encodes PNMT and alters epinephrine synthesis, it is possible that there is an interaction

between genetic variation of the ADRB2 and PNMT in the ventricular response to exercise (Table 5). For instance, within a given PNMT polymorphism, the Arg16 genotype can be hypothesized to have a lower stroke volume with exercise when compared with the Gly16 genotype. Also, an individual with the Arg16 genotype that also has the PNMT polymorphism that is known to have enhanced epinephrine synthesis likely has a higher stroke volume than an individual with the Arg16 genotype who carries the polymorphism of PNMT with attenuated epinephrine synthesis, simply because of decreased levels of circulating epinephrine. Of course, this relationship could be far more complex when taking into consideration possible differences in receptor desensitization, rather than simply baseline receptor number or function, because of genetic variation of PNMT and differences in epinephrine availability. Although studying the importance of gene-by-gene interactions and exercise is difficult because of the large number of subjects that is needed to obtain large enough sample sizes for a meaningful statistical analysis, this work is certainly possible if the correct proteins (those that play a large role in the cardiovascular or pulmonary response to exercise) and correct variants (those that are common and functional) are selected.

CONCLUSIONS

There have been hundreds of studies exploring the phenotypic consequences of variation in the gene that encodes the ADRB2. The sustained interest in the ADRB2 gene is warranted because of the ubiquitous nature of this receptor and a clear functional effect of variation in this gene. The previous studies in humans suggest that the Gly16Glu27 haplotype represents one that demonstrates a more favorable response to exercise when compared with the Arg16Gln27 haplotype. This is evident because the Gly16-Glu27 group demonstrates greater stroke volume, cardiac output, and enhanced vasodilation when compared with the Arg16Gln27 haplotype. In addition, the Gly16 group has demonstrated an enhanced ability to clear fluid from the lungs and sustained bronchodilation after short-term exercise, both of which may play a larger role with heavy and prolonged exercise. Although there is an interest in further exploring haplotypes of the ADRB2, research may be better spent on exploring the interaction between variation in this gene and variation in the genes encoding other adrenergic receptors and other proteins that are important in the cardiovascular and pulmonary responses to exercise.

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TABLE 1

Adrenergic receptors.

Adrenergic Receptor	Binds (Primarily)	Regulates
ADRB1	Norepinephrine	↑Cardiac inotropy, ↑heart rate, ↑lipolysis
ADRB2	Epinephrine	↑Glycogenolysis, airway/vascular smooth muscle relaxation, ↑cardiac inotropy
ADRB3	Norepinephrine	↑Lipolysis
ADRA1	Norepinephrine	Vasoconstriction, cardiac inotropy
ADRA2	Epinephrine	Presynaptic and postsynaptic transmission, vasoconstriction

ADRB1 indicates β_1 adrenergic receptor; ADRB2, β_2 -adrenergic receptor; ADRB3, β_3 -adrenergic receptor; ADRA1, α_1 -adrenergic receptor; ADRA2, α_2 -adrenergic receptor.

TABLE 2
Common sequence variants of the ADRB2 commonly combined to form haplotypes.

Haplotype	Codon (Amino Acid)						Haplotype Frequency ^a
	-654	46 (AA16)	79 (AA27)	252	164	175	
1	A	A	C	A	C	A	0
2	A	A	C	G	C	C	0.35592
3	A	A	G	G	C	C	0.00888
4	A	G	C	A	C	A	0.00225
5	A	G	G	G	C	C	0.00453
6	G	A	C	A	T	A	0.00221
7	G	A	C	G	C	C	0.02015
8	G	G	C	A	C	A	0.13492
9	G	G	C	A	C	C	0.03319
10	G	G	C	G	C	C	0.00446
11	G	G	G	A	C	A	0
12	G	G	G	G	C	C	0.43349

^a Actual data from Rochester, MN cohort. Sequence variants from multiple sites are often combined to form haplotypes and haplotype pairs, thus incorporating more genetic information. For example, haplotype 2 would be labeled as AACGCC for variation in the gene that encodes the β_2 -adrenergic receptor (ADRB2) at positions -654, 46, 79, 252, 164, and 175, respectively. With respect to positions 46 and 79 (amino acids 16 and 27, respectively), A encodes for arginine at position 16 and C encodes for glutamine at position 27.

TABLE 3

Frequency and cardiopulmonary function according to sequence variation of the ADRB2 at amino acids 16 and 27.

Codon	Substitution	Frequency (%)	Function
46	Arg16Gly	AA = 15, AG = 47, GG = 39	GlyGly more prevalent in hypertensives (2)
46	Arg16Gly	AA = 22, AG = 41, GG = 37	CHF patients with Gly allele have better pulmonary function compared with Arg (25)
46	Arg16Gly		GlyGly more receptors, greater cardiac function, airway function, natriuretic function, and lung fluid clearance (24–27,29)
46	Arg16Gly		GlyGly greater cardiac response to isometric handgrip (6)
46	Arg16Gly		GlyGly sustained response to infused isoproterenol (12)
79	Gln27Glu	GlnGln = 32, GlnGlu = 53, GluGlu = 16	GluGlu more prevalent in hypertensives (2)

ADRB2 indicates β_2 -adrenergic receptor; CHF, congestive heart failure.

TABLE 4

Proposed interaction between genetic variation of ADRB1 and ADRB2 and the cardiovascular response to exercise.

ADRB1 Genotype	Arg389		Gly389	
ADRB2 Genotype	Arg16Arg	Gly16Gly	Arg16Arg	Gly16Gly
Heart rate	= (↓)	= (↑)	= (↓)	= (↑)
Stroke volume	↑(↓)	↑(↑)	↓(↓)	↓(↑)
Cardiac output	↑(↓)	↑(↑)	↓(↓)	↓(↑)

Assuming Arg389 genotype has better β_1 -adrenergic receptor (ADRB1) function when compared with Gly389 genotype. Data are relative to ADRB1 genotype counterpart (relative to β_2 -adrenergic receptor (ADRB2) genotype counterpart, in parentheses).

TABLE 5

Proposed interaction between genetic variation of PNMT and ADRB2 and the cardiovascular response to exercise.

PNMT 5FR Haplotype	2*1		2*7	
ADRB2 genotype (amino acid 16)	ArgArg	GlyGly	ArgArg	GlyGly
Synthesis (Epi/NE)	↑ (=)	↑ (=)	↓ (=)	↓ (=)
Stroke volume (mL per beat)	= (↓)	= (↑)	= (↓)	= (↑)

Assuming the 2*1 haplotype of the phenylethanolamine N-methyltransferase (PNMT) demonstrates higher epinephrine levels relative to norepinephrine availability when compared with the 2*7 haplotype. Data are relative to PNMT genotype counterpart (relative to β_2 -adrenergic receptor (ADRB2) genotype counterpart, in parentheses).