Very important pharmacogene summary *ADRB2*

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The beta2-adrenergic receptor (beta2-AR) is a member of the G-protein-coupled adrenergic receptor family with seven transmembrane segments. Similar to other members of this receptor family, beta2-AR specifically binds and is activated by the endogenous class of ligands known as catecholamines, and epinephrine in particular. The gene encoding this receptor, *ADRB2*, was cloned by Kobilka *et al.* in 1987 and is localized to chromosome 5q31–q32, a region that has been linked with asthma and asthma related phenotypes [1,2]. *ADRB2* consists of a single exon of 2015 nucleotides, which encodes a 413 amino acid protein. This review highlights the genetic polymorphisms in *ADRB2* and the pivotal role of beta2-AR in the regulation of the cardiac, pulmonary, vascular, endocrine, and central nervous systems.

ADRB2 is abundantly expressed in bronchial smooth muscle cells and activation of the resulting receptor leads to bronchodilation. In addition, this gene is expressed in cardiac myocytes and vascular smooth muscle cells. Activation of beta2-AR in these cells causes an increase in the rate and force of heart contractions. Intracellular signaling upon beta2-AR activation is largely affected through a trimer of G proteins coupled to adenylate cyclase, to produce cyclic adenosine monophosphate. This, in turn, activates protein kinase A, leading to the phosphorylation and down-regulation of proteins including beta2-AR itself (please refer to PharmGKB β-agonist and β-blocker Pathway for further details: *https://www.pharmgkb.org/*do/serve?objId = PA2024&objCls = Pathway#).

Beta2-AR is the target of clinically important drugs for asthma and cardiovascular conditions including hypertension and congestive heart failure (CHF). Beta-receptor agonists (e.g. albuterol, salmeterol) and antagonists (e.g. carvedilol and propranolol) are among the most commonly prescribed medications in the treatment of asthma and cardiovascular disease, respectively. Although some beta-blockers are 'selective' for the beta1-AR (e.g. metoprolol and atenolol), these also antagonize the beta2-AR at higher concentrations. A number of genetic polymorphisms in the *ADRB2* gene have been described which affect gene expression, the function of the resulting receptor, and response to beta2-agonists.

ADRB2 variants

The *ADRB2* gene has been resequenced in multiple populations and more than 80 polymorphisms have been identified, of which 45 single nucleotide polymorphisms (SNPs) and two insertion/deletion polymorphisms have been validated in more than one study [3,4]. Two of these nonsynonomous SNPs code for amino acid changes at positions 16 [arginine to glycine (Arg16Gly); rs1042713] and 27 [glutamic acid to glutamine (Glu27Gln); rs1042714], are common with minor allele frequencies (MAF) between 40–50% and have been well characterized in asthma pharmacogenetics [5]. In-vitro studies showed that the Gly16 isoform enhanced the agonist-stimulated down-regulation of beta2-AR, whereas the Glu27 variant did not regulate the expression of this receptor [6,7]. In addition to these common polymorphisms, other less common, nonsynonymous coding variants have also been reported in the *ADRB2* gene. For example, the SNP rs1800888 encodes a Threonine to Isoleucine substitution at amino acid position 164 (Thr164Ile) and occurs with a MAF of 1–3%. The Ile164 isoform is three-to-four times less responsive to agonist-induced stimulation than carriers of the wild-type Thr164 [8,9]. Another rare, nonsynonymous variant resulting in a Valine to Methionine change at amino acid position 34 (Val34Met) in beta2-AR has a MAF less than 1% [10].

In-vivo studies of the genetic variants in *ADRB2* suggest that these are not likely to be disease-causing variants but possibly serve as predictive markers for responsiveness to both agonists and antagonists. Moreover, three meta-analyses of the two common nonsynonymous SNPs in *ADRB2* have concluded that these polymorphisms are not associated with the diagnosis of asthma [11–13]. However, homozygotes of Arg16 treated with regular short acting beta-agonist (SABA) therapy tend to experience

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more adverse effects [14]. Furthermore, among the patients prescribed beta-blocker therapy after an acute coronary syndrome, those homozygous for both Arg16 and Gln27 were at higher risk for death in 3 years (3-year mortality rate of 20%) compared to the other diplotypes (3-year mortality rate of 6–11%) [15]. CHF patients with the Ile164 variant were at higher risk for death or heart transplantation in 1 year (event rate 76%) compared to those homozygous for Thr164 whereas others did not observe this finding [16,17].

Important Variants (for full mapping information, see *http://www.pharmgkb.org*/search/annotatedGene/adrb2/variant.jsp)

(1) ADRB2: Arg16Gly; 285A > G (rs1042713),
(2) ADRB2: Gln27Glu; 318C > G (rs1042714),
(3) ADRB2: Thr164Ile; 730C > T (rs1800888).

ADRB2: Arg16Gly; 285A > G (rs1042713)

Arg16Gly is encoded by a common nonsynonymous polymorphism in the *ADRB2* gene. The estimated frequency of the Arg16 variant is 39.3% in White Americans, 49.2% in Black Americans and 51.0% among Chinese [10]. In-vitro studies using Chinese hamster fibroblasts showed that the Gly16 receptor had an enhanced agonist-promoted down-regulation relative to Arg16 [6]. Similar findings were reported for human smooth muscle cells [7]. Owing to the functional significance and the prevalence of the Arg16Gly variant, it has been the focus of many clinical studies on asthma and cardiovascular diseases. Three meta-analyses have shown that the Arg16Gly variant is not associated with asthma [11–13]. However, the allele encoding Gly16 has been associated with nocturnal asthma and with severe asthma [12]. Pharmacogenetic studies have observed an association between this polymorphism and response to beta2-agonists. Several studies have shown that homozygotes of Arg16 are more likely to respond (more rapid response and increased forced expiratory volume in one second) to albuterol (SABA) compared to homozygotes of Gly16 and heterozygotes [18–20]. One study observed this association only in response to high doses of SABA [21]. Other investigations, however, found no association between this SNP and variable drug response [3,22,23] whereas some groups reported contradictory results [24–26]. Individuals who are homozygous for Arg16 and receiving regular albuterol treatment reported to have decreased response, measured by lower morning peak flow rates, compared with those who were not receiving regular albuterol treatment, suggesting that regular albuterol therapy may not be appropriate for Arg16 homozygous asthma patients [27].

The Arg16Gly amino acid substitution has been shown to influence agonist-mediated vascular response. The allele encoding the Arg16 receptor was associated with enhanced isoproterenol-mediated vascular desensitization in a study involving 26 healthy volunteers [28]. This prospective study suggests that this isoform is an important determinant of the vascular response to stress [28]. In addition, effects of common beta2-AR haplotypes on vascular responses to a beta2-agonist have been studied in 35 healthy volunteers [29]. In this study, the Arg16 receptor showed higher sensitivity to terbutaline than the Gly16 isoform at baseline. After terbutaline treatment for 2 weeks, the extent of desensitization of venous beta2-AR differs by haplotype; Arg16Gln27Thr164 has the greatest desensitization whereas Gly16Glu27Thr164 showed the lowest desensitization [29]. However, these studies involved a small number of healthy volunteers who may have different physiology from that of patients with cardiovascular disease. In addition, this study did not randomize the treatment sequence to minimize the effects of the time. In another study, the Arg16 isoform was associated with higher peak oxygen consumption (peak VO_2) compared to Gly16 in 118 heart failure patients [30]. However, in another cohort study of 199 patients with stable CHF, the Arg16 isoform was not associated with improvement of left ventricular ejection fraction and decrease in heart rate in response to a β-blocker [31]. In a cohort study with 171 idiopathic dilated heart failure patients, the Arg16 isoform was associated with lower risk of death or heart transplantation compared with the Gly16 [32]. However, these findings have not been replicated. In fact, studies have produced conflicting results regarding an association between beta2-AR haplotypes and death or heart transplantation in stable heart failure. Although homozygosity for Arg16Gln27 haplotype was associated with an increased risk of death or heart transplantation in a prospective cohort study involving 227 patients [33], no beta2-AR haplotypes were associated with the outcomes in another prospective cohort study of 637 patients [34]. The studies evaluating intermediate or clinical outcomes have relatively small sample sizes and have different rates of background medications such as angiotensin-converting enzyme inhibitors and β-blockers for heart failure, which may account in part for the conflicting results.

Association studies of the Arg16Gly substitution with type-2 diabetes mellitus [35,36] and risk factors such as obesity, hypertension and insulin resistance have also reported conflicting results. A nominal association with the Arg16 variant in type-2 diabetes was found in a case-control study of 7808 unrelated, middle-aged White populations [36]. In another study of 130 Taiwanese patients with type-2 diabetes matched 1 : 1 for sex, age, and body mass index (BMI), two copies of the Arg16 isoform was an independent risk factor for development of type-2 diabetes and was associated with earlier disease onset [37]. However, there are other studies reporting Gly16 as the risk variant. The affect of this polymorphism on insulin secretion was studied in a cohort of 47 Japanese type-2 diabetic patients. Gly16 homozygotes

had significantly higher levels of fasting insulin and homeostasis model assessment of insulin resistance compared with the Arg16 homozygotes [38]. These findings are in agreement with similar studies where the Gly16 isoform was associated with higher insulin resistance in nonobese, normotensive Japanese individuals [39]. Likewise, conflicting associations have been reported for hypertension risk among type 2 diabetic patients, with some groups reporting increased risk of hypertension associated with Arg16 [40] and others reporting associations with the Gly16 isoform [41]. Finally, several studies have reported increased BMI correlated with the Arg16 isoform [41–43], while other studies found a protective effect of the same allele [37,40]. A meta-analysis of 11 populations from earlier studies reported no association between the polymorphism encoding Arg16 and obesity [44].

Earlier studies have suggested that the Arg16Gly variant may be associated with cholesterol metabolism in certain populations. A study of 100 hypertriglyceridemia cases and 241 healthy controls, from a population of Chinese Han showed that controls who were homozygous for the Arg16 isoform had higher serum triglycerides. In hypertriglyceridemia patients, Arg16 homozygotes had higher serum total cholesterol and low-density lipoprotein cholesterol levels (207.27 ± 28.62 vs. 184.46 ± 41.38 mg/dl, $P < 0.05$; 117.17 ± 27.07 vs. 92.03 ± 42.54 mg/dl, $P < 0.05$) [45].

The impact of the Arg16Gly amino acid substitution on other cardiovascular outcomes such as sudden cardiac death, ventricular arrhythmias, myocardial infarction (MI), and stroke has also been studied. Case-control studies reported no association between this polymorphism and the above-mentioned phenotypic outcomes [46,47]. Although these case-control studies have relatively large sample sizes (495–5393), they may have been confounded by unmeasured factors. Overall, data on the association of the Arg16Gly isoform with clinical outcomes in cardiovascular diseases are not consistent. Therefore, more studies involving larger sample sizes and a better design are needed to define the roles of this polymorphism in cardiovascular diseases.

ADRB2: Gln27Glu; 318C>G (rs1042714)

Gln27Glu is encoded by a common nonsynonymous polymorphism (rs1042714) in the *ADRB2* gene. The estimated frequency of the Glu isoform is 24.6% among Whites, 18.7% among Blacks, and 9% among Chinese [10]. Earlier studies have suggested that the Glu27 isoform do not down-regulate the expression of the beta2-AR [6,7]. Individuals who were homozygous for Glu27 had higher maximal venodilatation in response to isoproterenol than those who were homozygous for Gln27, suggesting that the Gln to Glu change is associated with increased agonist-mediated responsiveness [28]. Impact of the *ADRB2* polymorphisms on vascular responses to isoproterenol were studied with internal mammary arteries obtained from 96 patients undergoing coronary bypass surgery. The arteries from patients homozygous for Gly16 displayed reduced sensitivity to isoproterenol compared with those from patients carrying Arg16. Among the arteries from the Gly16 homozygotes, those from the patients homozygous for Glu27 showed isoproterenol sensitivity similar to the arteries from the Arg16 carriers [48]. Thus, overall data suggest that the *ADRB2* polymorphisms may influence vascular responses to a beta2-agonist. In addition, the Gln27 receptor has been associated with increases in systolic blood pressure [49].

Several studies found that the beta2-AR mediated increases in heart rate and contractibility are not dependent on the amino acid changes at codons 16 and 27 [9]. The polymorphism encoding the Gln27Glu change was not associated with the increased risk of sudden cardiac death and ventricular arrhythmias in patients with coronary artery disease [46], nor is it associated with the risk of MI or ischemic stroke in patients who were pharmacologically treated for hypertension [47]. The polymorphism has also been studied in heart failure. In a prospective cohort study with 80 patients with heart failure, those homozygous for Gln27 were less likely to have improved left ventricular ejection fraction after carvedilol treatment compared to Glu27 carriers [50]. However, in another prospective cohort study with 199 heart failure patients, this variant was not associated with the improvement of left ventricular ejection fraction or decrease in heart rate in response to a β-blocker [31]. Nevertheless, the Gln27 isoform was associated with a lower risk of death or heart transplantation in idiopathic dilated heart failure [32]. In addition, the Gln17 isoform, in the presence of the Gly16 and Ile164 variants were associated with decreased risk of MI [49]. Thus, data on the role of this polymorphism in heart failure are conflicting. In a prospective cohort study involving 735 patients who were prescribed a β-blocker after an acute coronary syndrome, patients homozygous for Gln27 had higher mortality rate (16%) compared to those heterozygous and homozygous for Glu27 (11 and 6%, respectively). In addition, those homozygous for both Arg16 and Gln27 were at higher risk for death in 3 years (3-year mortality rate 20%) compared to the other diplotypes (3-year mortality rate 6–11%) [15]. Although these findings have not been replicated, the Arg16Gln27 diplotype is associated with higher mortality in patients who receive a β-blocker after acute coronary syndrome.

Association studies of the Gln27Glu variant and type-2 diabetes mellitus have yielded neutral [35–37], positive [43,51], and contradictory [52,53] results in various populations. In a case-control study of 7808 unrelated, middle-aged Whites, no association was found with

obesity, hypertension and type-2 diabetes [36]. However, in another case–control study of 400 nonobese individuals (BMI $< 27\,kg/m^2$) and 108 obese individuals (BMI $\geq 27\,kg/m^2$), the frequency of the Glu27 variant was higher in type-2 diabetics than nondiabetic participants (0.14 vs. 0.07, $P=0.001$, odds ratio (OR): 2.13, 95% confidence interval 1.34–3.41) [43]. Conversely, in 342 type-2 diabetic patients and 305 unrelated nondiabetic controls, Glu27 homozygotes had a lower frequency of diabetes when compared to Gln27 carriers (OR: 0.56, 95% confidence interval 0.36–0.91) [52]. A study in 1054 Swedish participants with varying degrees of glucose tolerance had different findings. In 219 type-2 diabetic patients, the Gln27 variant was seen more frequently than in 237 matched nondiabetic participants (59.8 vs. 52.3%; OR=1.72, $P=0.02$). Glu27 homozygous individuals had the lowest prevalence of diabetes [53]. In a case-control genetic association study of 161 healthy Whites and 74 African–Americans, Gln27 homozygotes compared to Glu27 carriers tended toward higher insulin levels and greater insulin resistance as determined by homeostasis model assessment of insulin resistance [54]. Similarly, a cohort of 102 black South African women found that the Glu27 isoform was associated with higher insulin resistance among obese individuals [55].

Homozygotes of the Gln27 variant in the presence of Arg16 had increased risk of obesity [41], whereas the Glu27 was also associated with increased BMI in African–Americans and Hispanic Americans [56]. Another study reported that the Glu27 receptor was a risk factor for abdominal obesity among males, particularly among those with low HDL cholesterol [57]. A meta-analysis of 23 populations reported variable association results across the different populations, with a summary of OD showing no association between this polymorphism and obesity [44]. However, the prevalence of Glu27 ranged from 6.71 to 78.29% across the populations, so that the polymorphism encoding this isoform is significantly associated with obesity in race groups with low frequency of this allele such as Asians, Pacific Islanders, and American–Indians, but not European populations, in which this allele is highly prevalent.

A cohort of 1050 Whites were evaluated to determine if *ADRB2* polymorphisms would predict the occurrence of metabolic abnormalities in hypertensive patients given a β-blocker (atenolol 50–100 mg or metoprolol 100–200 mg daily) for 6 months. They found the Glu27 variant was associated with a higher incidence of dyslipidemia [58], which has been found by other groups as well [59] where heterozygous Gln27Glu hypertensive patients had an increase in triglyceride levels following use of 100 mg metoprolol daily for 2 months and also after use of propranolol in healthy individuals [60]. These data are similar to others, with others [61,62] who observed the same association of the Glu27 variant and hypertriglyceridemia.

ADRB2: Thr164Ile; 730C>T (rs1800888)

As mentioned in an earlier section, the Thr164Ile variant is less common than the Arg16Gly and Gln27Glu amino acid changes. The estimated frequency of the allele encoding the Ile164 isoform is 1% in Whites, less than 2% in Africans, and nonpolymorphic in Chinese [10]. Receptors containing the Ile164 variant showed a substantial decrease in basal and epinephrine-stimulated adenylyl cyclase activities because of defective coupling of the receptor to the stimulatory G protein, Gs, and impaired agonist-promoted sequestration. Ile164 also displayed a lower binding affinity for epinephrine as compared with the wild-type beta2-AR [8]. Consequently, this amino acid change has been associated with reduced response to the long-acting beta2-agonist salmeterol [8,63].

In-vivo studies found that an increase in heart rate and contractibility mediated by beta2-AR in response to terbutaline is blunted in individuals heterozygous for Ile164 compared with those homozygous for Thr164 [64]. A potential association between the Ile164 variant and hypertension was found only in women in a large cross-sectional study with 9185 participants [65]. The impact of the Thr to Ile change at codon 164 on death or heart transplantation in heart failure is not clear at this time because of conflicting results. A prospective cohort study of 257 patients found that those with the Ile164 variant were at higher risk for death or heart transplantation in 1 year (event rate 76%) compared to patients homozygous for Thr164 [16]. However, the Ile164 variant was not associated with these outcomes, but may interact with β-blocker treatment in a recent prospective cohort study with 443 heart failure patients [17]. The low frequency of the Ile164 isoform and unmeasured confounders may have contributed to the conflicting results. Impact of the Thr164Ile substitution on the outcomes in patients who received percutaneous coronary intervention has also been studied. In a prospective cohort study with 330 patients, those carrying the Ile164 variant were 3.7 times more likely to have cardiac death and 4.1 times more likely to have a major cardiac adverse event than patients homozygous for Thr164 after percutaneous coronary intervention [66]. The higher incidences of acute MI and a major cardiac adverse event have been replicated in a separate cohort of 150 patients with peripheral arterial disease [28]. Overall, the roles of the Thr164Ile variant in cardiovascular outcomes have not been well defined. Because of the low allele frequency, studies with larger sample sizes would help define the effect of this amino acid substitution on the clinical outcomes of cardiovascular diseases.

Conclusion

Variants in the *ADRB2* gene encoding beta2-AR have been correlated with variable response to drugs for asthma and cardiovascular medications as well as disease

risks such as type-2 diabetes, obesity and hypertension. However, the directions of these correlations differ across studies and remain to be replicated in larger studies. A meta-analysis by Contopoulos-Ioannidis *et al.* reported that most associations between the two common polymorphisms in *ADRB2* and asthma drug response and other asthma related phenotypes are statistically insignificant because of small sample sizes and less than 2% of the associations were replicated by two or more groups [67]. In addition, correlations between these variants and beta2-agonists may be specific to short-acting beta2-agonists, and not affect response to long-acting drugs. Pharmacogenetic correlations may also be affected by the interval of drug treatment (regular use or use as needed) and interactions with other medications. Furthermore, these associations may also be specific to certain ethnicities and subject to sex effects. Cagliani *et al.* described ethnicity-specific and sex-based haplotype distributions of the *ADRB2* variants [68]. Similar findings were reported in a meta-analysis by Jalba *et al.* which resulted in differences in association across populations [44]. Moreover, the relative fitness associated with these haplotypes varies under the influence of epistasis and imprinting. Experiment techniques that can directly access the functional importance of beta-adrenoceptor polymorphisms on ligand-induced conformation changes (e.g. fluorescence resonance energy transfer) will also help clarify the discrepancies with respect to the role of these polymorphisms in disease susceptibilities and therapeutic responses [69].

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