Exploration of the β2-adrenergic receptor regulatory regions: the next step in the holy grail of asthma pharmacogenetics research

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EXPLORATION of the β2-adrenergic receptor (ADRB2) has been the holy grail of asthma pharmacogenetics research since the gene was cloned two decades ago. The evolution in our understanding of genetic variants in the ADRB2 reflects both the progress and the limitations in exploring the contributions of an important candidate gene within a complex phenotype such as asthma.

As the G protein-coupled receptor that is the direct target of β2-agonist medications, ADRB2 has been highlighted as a promising candidate gene linked to asthma. Identification of single nucleotide polymorphisms (SNPs) within the 1,242-bp coding block proved relatively easy in this intronless gene (14). Seven coding block polymorphisms are present with minor allele frequency >1–2%, but only two of these genetic variants confer amino acid changes: at codons 16 and 27 in the amino terminus of the receptor. Studies of linkage disequilibrium (LD) in different populations have demonstrated that the Arg16 allele is nearly always in LD with the Gln27 allele so that the combination of these SNPs produces only three haplotypes: Arg16Gln27, Gly16Glu27, and Gly16Gln27. Neither the coding block polymorphisms nor their associated haplotypes have been associated with the diagnosis of asthma per se in multiple studies of asthma populations (2, 14).

The early in vitro studies using Chinese hamster fibroblasts and human airway smooth muscle cells suggested that these polymorphisms had functional significance and helped to establish the dogma that Gly16 is associated with increased desensitization after exposure to β-agonists (4, 5). Despite a number of clinical studies, there has been no consensus on which polymorphism influences asthma severity (15). However, chronic β-agonist use may be important in individuals homozygous for the Arg16 allele. Two recent studies from the Asthma Clinical Research Network (BAGS and BARGE) report that patients homozygous at Arg16 experienced a worsening in their lung function (morning peak expiratory flow) while on regularly scheduled albuterol (7, 8). These observations have been validated in studies of long-acting β-agonists (11, 17), but other recent studies of Arg16 have shown no relationship with this phenotype (1). The in vitro basis for this effect of Arg16 has not been established but could be related to LD with polymorphisms in the regulatory regions.

Our understanding of the regulatory regions of the ADRB2 gene has evolved slowly. An initial study suggested that a polymorphism 47 bp upstream of the translation start site may influence ADRB2 expression (13). The ADRB2 transcription start site is 5’ to a small open reading frame that encodes a 19-amino acid peptide (denoted as the β2-adrenergic receptor upstream peptide, BUP), and this polymorphism results in a Cys to Arg substitution in the 19th amino acid of the BUP. The BUP has been shown to modulate receptor translation, and the Arg19 BUP was associated with decreased receptor expression (9). The genetic variants that encode the BUP and amino acid codon 27 are in complete LD, so it is possible that any association with Glu27 or Gln27 may be a proxy for functional genetic variants in the promoter region.

Liggett’s group (3) expanded their search of the regulatory regions and resequenced the ADRB2 gene from ~1,100 bp upstream through ~700 bp of the coding block, using a repository of DNA from 77 individuals from four ethnic backgrounds. Their analysis of the ADRB2 gene revealed that four extended haplotypes account for >90% of the persons genotyped from each of the four ethnic backgrounds. Hawkins et al. (6) resequenced a 5.3-kb region in 429 whites and 240 African Americans and identified 31 SNPs with minor allele frequency >3%, including 22 SNPs in the promoter region. Even with this number of SNPs, because of such strong LD across this region, this more extensive resequencing confirmed the presence of only four extended promoter/coding block haplotypes that account for the vast majority of persons genotyped. Table 1 demonstrates the allele frequencies of these four extended haplotypes, numbered as per the original report of Drysdale et al. (3).

The coding block haplotypes Gly16Gln27 (haplotype 6) and Gly16Glu27 (haplotype 2) are each associated with their respective promoter haplotypes; in contrast, the coding block haplotype Arg16Gln27 is associated with two different promoter haplotypes (haplotypes 1 and 4), one of which (haplotype 1) is found only in African Americans. Perhaps difficulty in replicating positive associations of the Arg16Gln27 haplotype may in part be explained by relative differences in frequencies of the respective promoter haplotypes within a given cohort. In Table 1, the four 5’ SNPs listed are representative in that they are in LD with the other SNPs in the promoter region.

This “clustering” of haplotypes gives an overview of the relative frequencies of the majority of SNPs, but the contribution of rare SNPs with functional significance may be missed. Clinical studies of short- and long-acting β2-agonists have suggested that there are individuals who are prone to poor response or desensitization, which might be explained by rare SNPs in key regulatory regions of the ADRB2 gene. In their comprehensive resequencing and analysis, Hawkins et al. (6) identified a number of rare genetic variations in regulatory regions, although the clinical relevance of these has not been established. In addition, sequencing of the 3’-untranslated region (UTR) revealed a poly-C repeat starting 23 bp after the TAA stop codon and varying in size from 9 to 15 Cs. In their analysis, the length of the poly-C repeat was associated with lung function [forced expiratory volume in 1 s (FEV1)] and % predicted forced vital capacity (FVC) in African Americans (6). This poly-C region lies only 20 bp 5’ of an AU-rich
In their article in the American Journal of Physiology-Lung Cellular and Molecular Physiology, Panebra et al. (12) utilize an in vitro model to explore the significance of this poly-C repeat. BEAS-2B cells were transfected with constructs containing ADRB2 followed by its 3′-UTR with 11, 12, or 13 Cs within the poly-C tract. They demonstrate that ADRB2-11C had lower ADRB2 mRNA and protein expression, which appeared to be the result of reduced RNA stability. After 12 h of exposure to the agonist isoproterenol, there was 50% less downregulation of ADRB2-12C compared with ADRB2-11C or ADRB2-13C. The authors suggest that together these data suggest that ADRB2-11C may be a less responsive phenotype (lower basal expression, more easily downregulated) and that ADRB2-12C may be a more responsive phenotype (higher basal expression, less easily downregulated).

Throughout the evolving ADRB2 story, Liggett’s group has consistently provided in vitro data to provide a mechanistic basis for the genetic association studies. On the basis of these provocative observations, the length of this poly-C repeat should be included as another marker in future pharmacogenetic studies involving ADRB2. There are some caveats about whether these specific in vitro findings will be generalizable. These constructs were made by using the Arg16 coding sequence followed by the 3′-UTR including the poly-C tract. Although this in vitro system allows one to study the effect of variation in the poly-C tract in isolation, there are a number of other regulatory elements in the promoter region that may also alter ADRB2 expression in vivo. In their analysis, Hawkins et al. noted the association of the poly-C repeat with lung function only in African Americans, and yet the frequency of the longer (more responsive) tracts is higher in African Americans (frequency of 13C = 0.64) (6). Nevertheless, inclusion of the poly-C tract with polymorphisms in other regulatory regions of the ADRB2 gene may actually give a stronger signal in future genetic association studies.

Where do these results leave us in our search for the holy grail? They are a reminder that regulatory elements in both the 3′-UTR and 5′ regions may have more functional significance than the coding block polymorphisms, and that the inability to replicate associations with specific polymorphisms may reflect partial linkage with other regulatory elements. And, just as a single polymorphism does not sit in isolation but is part of a larger haplotype family, the ADRB2 gene does not sit in isolation. Genetic variation in any of the genes in the ADRB2-containing ADRB2 followed by its 3′-UTR downstream peptide. Adapted from Drysdale et al. (3) and Hawkins et al. (6).

**Table 1. β2-Adrenergic receptor polymorphisms and haplotypes**

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<td>A216 Gly/Arg</td>
<td>A27 Gln/Glu</td>
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BUP, β-adrenergic receptor upstream peptide. Adapted from Drysdale et al. (3) and Hawkins et al. (6).

...contains ADRB2 followed by its 3′-UTR, and viral infection (10, 16, 18).

REFERENCES


