Polymorphic Variation in the G-Protein Beta-3 Subunit Gene and Response to BiDil in A-HeFT

Basis for an African-American Pharmacogenetic Advantage to Nitric Oxide Donor Therapy?*

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Guanine nucleotide-binding proteins, or G-proteins, typically provide the major signal transducing step between agonist occupancy of a 7-transmembrane receptor and effector mechanisms. G-proteins consist of 3 subunits (α, β, and γ); the β and γ subunits are tightly bound and typically function as a “βγ” subunit. Both the α and βγ subunits serve signal transduction functions. G-protein–coupled receptor (GPCR) activation catalyzes guanosine diphosphate (GDP)–guanosine triphosphate (GTP) exchange that results in temporary dissociation of α and βγ subunits, and each is then free to effect downstream signaling via protein–protein interactions. Signal transduction via free/activated α subunits is well known (e.g., stimulation and inhibition of adenylyl cyclase). βγ–subunit-mediated signal transduction was appreciated later but is also important and includes muscarinic activation of cardiac Kir3.1 channels (1), β-adrenergic activation of G-protein-coupled receptor kinase-2 (GRK2) (2), and GPCR responses known to be operative in vascular smooth muscle (3–5).

In humans there are 16 (6), 5 (7), and 12 (7) different genes coding α, β, and γ proteins, respectively. Mammalian β1 to β4 subunits have high amino acid homology and likely arose from a common ancestor, whereas β5 is less similar (~50% homology to β1 to β4) (7). The large number of potential βγ-subunit combinations implies the potential for substantial diversity of downstream signaling, but the details of how the various βγ-subunit combinations engage in different effector targeting is not well understood (7).

In 1998, Siffert et al. (8) reported a polymorphism in the β3 subunit that was present in transformed lymphoblasts from hypertension patients but not in normal controls, which conferred elevated sodium/hydrogen (Na⁺/H⁺) exchanger activity. This variant consisted of a synonymous single nucleotide polymorphism (SNP) C825T in exon 10 of the β3 gene (officially named GNB3) that was associated with the production of a splice variant, β3-s, from the 825T allele. Nucleotides 498 to 620 (123 bp) in exon 9 were deleted, resulting in a protein with a deletion of 41 amino acids. The amino acid deletion was in the functionally important “WD” (consisting of tryptophan-aspartic acid repeats) region that forms tight binding with γ subunits, and the β3-s variant exhibited greater functional signal transduction in model systems (8). Siffert’s laboratory also identified another gain-of-function GNB3 splice variant polymorphism associated with the 825T allele, β3-s2, which has a 129 bp deletion in exon 9 that results in loss of one WD domain similar to β3-s (9).

Since this first report of an association of the GNB3 825T allele with hypertension (8), multiple studies have found a relationship between GNB3 polymorphisms and hypertension or other cardiovascular disease risk, as cited in McNamara et al. (10) in this issue of JACC: Heart Failure. These authors (10) reported data from a DNA substudy of the A-HeFT study.
(African American Heart Failure Trial) (11), which was conducted exclusively in African Americans with heart failure and reduced left ventricular ejection fraction (HFrEF). In this A-HeFT DNA substudy (10), composed of 33% of the total number of enrolled patients, the effectiveness of fixed-dose hydralazine/isosorbide dinitrate (BiDil) was enhanced in patients who were \textit{GNB3} 825T homozygotes compared with 825C allele carriers. For the A-HeFT primary endpoint, a weighted composite of death, heart failure hospitalization, and change in quality of life (11), patients with a \textit{GNB3} 825T homozygous genotype who were receiving BiDil had a 0.61-unit improvement compared with placebo (p = 0.02), whereas 825C carriers had essentially no treatment effect (0.04-unit improvement, p = 0.87) (10). For comparison, the all-genotypes 350-patient DNA substudy parent population had a BiDil treatment effect of 0.33 units (p = 0.07), and the A-HeFT entire cohort of 1,050 patients had a composite endpoint treatment effect of 0.40 (p = 0.010) (11). Survival was also enhanced, with a 46% reduction (p = 0.047) in mortality at 18 months in the BiDil \textit{GNB3} 825T homozygous group, compared with a 23% (p = 0.35) increase in mortality in the 825C carrier BiDil group (10).

Based on previous work by other laboratories, a possible explanation offered for these findings is an amplified effect of nitric oxide (NO) donation in \textit{GNB3} 825T homozygotes (12,13) that countered enhanced vasoconstriction delivered by \(\beta_2\)-adrenergic receptor-mediated vasoconstriction (13,14). This explanation, as well as the primary \textit{GNB3} polymorphism findings, should be considered hypothesis generating, but these findings are certainly worthy of further study.

The A-HeFT DNA substudy has also provided evidence that an NO synthase (NOS3) (15) and an aldosterone synthase (16) polymorphism are each associated with an enhanced BiDil treatment effect; because of the relatively small sample size, a possible interaction with these variants and \textit{GNB3} was not investigated (10). For all 3 polymorphisms, the higher response variant is enriched in African Americans or in other populations of African descent (10,15–19). Therefore, it is possible that multiple racially distributed genetic variants contribute to the presumed higher efficacy of BiDil in populations of African ancestry (11,20). However, relative to Caucasians the racial enrichment of the higher response allele is far greater for \textit{GNB3}, by 2.1-fold (10) to 2.7-fold (17), compared with the other 2 candidates: 1.3-fold (18) to 1.4-fold (15) for NOS3 and 1.4-fold for the aldosterone synthase gene, \textit{CYP11B2} (16,19). These quantitative differences are in part the result of the minor allele status of \textit{GNB3} 825T; the other 2 African-American enriched high-response variants are major alleles, and the comparative increase in frequency for them therefore cannot be >2-fold. However, that does not negate the importance of a large relative change, particularly for an allele such as \textit{GNB3} 825T that confers major biologically relevant functional effects. Thus the available data would favor \textit{GNB3} polymorphic variation as the leading pharmacogenetic candidate for the apparent increased effectiveness of BiDil in African Americans. Although there are other hypotheses for why an NO donor therapeutic agent would have a preferential effect in African Americans (21), the A-HeFT \textit{GNB3} data justify a prospective test of the hypothesis that the ~50% of African-American HFrEF patients who are 825T homozygotes have a superior response compared with patients who are 825C carriers.

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