CHAPTER II:
IDENTIFICATION OF CANDIDATE GENETIC VARIANTS

Summary

Chapter I introduced the severity of the HF public health problem and the roles that the SNS and RAAS play in HF pathophysiology and clinical outcomes. The goal of this chapter was to identify candidate genetic variants within the SNS and RAAS that have *in vitro* and *in vivo* evidence to support an association with HF survival and beta-blocker response. A final list of eleven candidate genetic variants was identified in the literature and will be used for analyses. A comparison of the candidate gene approach to other genetic association approaches was introduced, and the limitations of the candidate gene approach used herein were discussed.

Introduction

Because there are approximately three million differences in DNA sequences between individuals, deciding which genetic variants to test and how many to test is an extreme challenge for investigators. Genetic association study designs fall on an enormous spectrum. On one extreme, an investigator may test a single genetic variant. On the other extreme, an investigator may scan the entire genome. When an investigator decides to only test one or a few variants, this is referred to as a "candidate gene approach" because the investigator has a strong *a priori* hypothesis for the best candidates to find an association. The *a priori* hypothesis for the candidate genetic

variants is ideally based on substantial pre-existing evidence for an association with the trait (e.g. molecular, physiologic, pre-clinical, or clinical evidence). When pre-existing evidence for particular candidate genes is lacking, an investigator may test all of the common variation in the genome. This is referred to as a genome-wide association study (GWAS), which has been referred to as "hypothesis-free," or the broad hypothesis that there are variants somewhere within the genome that will be associated with the trait. Many genetic association study designs fall somewhere in the middle of the two extremes, with intermediate numbers of genetic variants tested for a general hypothesis. For example, if the trait is drug response, the hypothesis could be that any genetic variation within pharmacokinetic genes (e.g. drug metabolizing enzymes and transporters) will be associated with drug response. This hypothesis is narrower than testing the entire genome, but it also does not make the hypothesis for specific variants. Instead of 1 variant or 3 million variants, this hypothesis falls in the middle range with a few thousand variants.

Of course each end of the genetic association spectrum (i.e. candidate gene association versus GWAS), has its advantages and disadvantages. When only selecting a few genetic variants, obviously genetic association from other genes will be missed. The primary limitation of candidate gene association studies is that they are limited to known or hypothesized biologic relevance. However there may be yet undiscovered genes important for a trait, which would be detected in the GWAS. For example, in another common, complex disease, age-related macular degeneration, candidate gene studies failed to find any genetic variants that accounted for a large proportion of the overall prevalence, but GWAS did (1). However a disadvantage of testing many genetic variants

is the potential for false positives. For example, if testing 1 million variants, and using an alpha = 0.05, the investigator may find 50,000 variants associated with the trait by chance alone. Therefore to control for the potential false positives, stringent levels for statistical significance must be set, and therefore very large sample sizes are required to have the power to detect true associations. In this sense, candidate gene studies have the advantage of being flexible and having power to test few variants. Another advantage of candidate gene studies is coverage of uncommon or rare variants. GWAS typically only cover common genetic variation (minor allele frequency [MAF] > 5%). Cost is also a significant factor when deciding on the number of variants to test, as genotyping costs and sample sizes must increase with the number of variants. GWAS are typically hypothesis generating and therefore require validation samples as well. In addition, the data generated in GWAS are computationally intensive and often require more elaborate IT infrastructure and statistical support than a candidate gene study.

The candidate gene approach was chosen for this dissertation research for several reasons. The sample size of the HF patient cohort (n = 720) was not amenable for GWAS, which requires thousands of patients for power to meet the stringent statistical significance thresholds. And also the lower cost of only genotyping a few variants was another reason. There is also good data in the literature to support an *a priori* hypothesis for certain variants. Specifically, it is well-established that the SNS and RAAS are involved with HF pathophysiology and pharmacology. There are known genetic variants in these systems that are known to affect these systems at molecular, physiologic, and clinical levels, and hence a "hypothesis-free" GWAS approach was not required.

The rationale for candidate variant selection is as follows: Because the SNS and RAAS are so closely associated with HF pathophysiology and pharmacology (See Chapter I: Introduction), variants affecting those systems were chosen. Variants also known to affect gene expression or function were chosen because they are more likely to translate into clinically relevant outcomes. Much variation in the genome is thought to be random mutation and have a neutral effect; this variation would not be expected to translate into clinically meaningful outcomes. Because the best candidates for an association with HF clinical outcomes (survival and beta-blocker response) wanted to be chosen, variants that had effects further translating from molecular effects to physiologic or clinical outcomes (e.g. ventricular remodeling or beta-blocker response) were chosen. Only common variants (e.g. MAF >5% in Caucasian-Americans or AAs) were chosen due to statistical power and generalizability of the results to large numbers of patients. Herein the methods for finding candidate genetic variants that meet these criteria were described and the literature supporting the candidate variant list was summarized.

Methods

Selection criteria. Candidate genetic variants must be part of the SNS or RAAS, have a MAF of greater than 5% in Caucasian-Americans or AAs, affect gene expression or function, and are associated with HF patient clinical outcomes or relevant physiologic processes (i.e. ventricular remodeling). When linkage disequilibrium exists, the true functional variant was chosen if known.

Search strategy. Candidate genetic variants were identified in the PubMed database from 1966 to May 2009 by combining the following search terms: heart failure,

sympathetic adrenergic system, renin-angiotensin-aldosterone system, genetic, polymorphism, beta-blocker, pharmacogenetic, survival, and left ventricular ejection fraction. Once variants were identified to have an association with HF relevant outcomes such as drug response, ventricular remodeling, or survival, then functional and pre-clinical data was searched for using the specific variant name or rsID (reference sequence identifier). Variant population frequencies were found in published literature, the HapMap database (2), or NCBI (3). Studies were limited to those published in English.

Results

Twelve candidate variants met the selection criteria. However one candidate variant, M235T in the gene for angiotensinogen (*AGT*), was excluded because it was found to be in complete linkage disequilibrium with G-6A in *AGT*, which was later determined to be the causal functional variant (4). Table 1 summarizes the identification and location information for the candidate genetic variants. Table 2 summarizes the MAF of the variants in populations of Caucasian and African descent. Tables 3 & 4 summarize the gene function within SNS or RAAS, respectively, and the molecular and clinical HF phenotypes of the variants.

Discussion

Eleven candidate genetic variants in the SNS and RAAS were chosen for this dissertation research based on candidate frequency and molecular/clinical phenotypes. It is important to point out the limitations of this candidate gene approach. Given the complexity of the SNS and RAAS, it is somewhat surprising that only 11 candidate

genetic variants met the selection criteria. Therefore important genetic variation in these systems may be missed in this dissertation research simply because they have not been previously studied. This also highlights the fact that genetic variation in systems other than the SNS and RAAS, such as inflammatory, that affect the HF phenotype are also not being tested as part of this dissertation research. Importantly, when looking at beta-blocker response in HF, no pharmacokinetic (PK) genes were selected for this dissertation. However the few studies testing for an association between PK genetic variants and beta-blocker response have not found an association with pharmacodynamics (5,6). Publication bias, in which the effects of a variant may be false positive or exaggerated (7), is also an important consideration because the variant selection was based on previously published literature. The genetic and pharmacogenetic literature for HF is still in very early stages, as there is not a great deal of literature available, and the current literature covers only a few variants in small sample sizes. Of course the candidate gene approach is not the only method for finding genetic association. An alternative method would be to use tag SNPs to cover all common genetic variation in the SNS or RAAS genes, instead of just picking only one or two variants. Of course GWAS is another alternative, because in a complex disease such as HF, many genetic variants are probably involved. However, a large HF patient population would be required. The strengths of the 11 candidate genetic variants for the dissertation research are low cost, low computational demand, improved statistical power, frequency, and substantial background *in vitro* and *in vivo* data.

Table 1. Identification and location information of candidate genetic variants

Gene	rsID	Nucleotide substitution	Amino acid Substitution	Chromosome	Chromosome position (GRCh37.p5 assembly)	Variant type
ADRB1	rs1801252	A1231G	Ser49Gly	10	115804036	missense SNP
ADRB1	rs1801253	C1251G	Arg389Gly	10	115805056	missense SNP
ADRB2	rs1042713	A285G	Arg16Gly	5	148206440	missense SNP
ADRB2	rs1042714	C318G	Gln27Glu	5	148206473	missense SNP
ACE	rs1799752	287-bp deletion	n/a	17	61565890	intronic indel
ADRA2C	rs61767072	12-bp deletion	322GlyAla-GlyPro325	4	3769297	frameshift indel
GRK5	rs17098707	A355T	Gln41Leu	10	121086097	missense SNP
AGT	rs5051	G-6A	n/a	1	230849872	5’ UTR SNP
AGTR1	rs5186	A1166C	n/a	3	148459988	3’ UTR SNP
CYP11B2	rs1799998	C-344T	n/a	8	143999600	5’ UTR SNP
BDKRB2	n/a	9-bp deletion	n/a	14	n/a	non-coding exon indel

Table 2. Minor allele frequencies (MAF) of candidate genetic variants

Gene	rsID	Minor allele	MAF Caucasian	MAF African
ADRB1	rs1801252	G	0.17	0.25
ADRB1	rs1801253	G	0.27	0.38
ADRB2	rs1042713	A	0.40	0.50
ADRB2	rs1042714	G	0.42	0.20
ACE	rs1799752	Ins	0.44	0.43
ADRA2C	rs61767072	Del	0.04	0.43
GRK5	rs17098707	T	0.02	0.24
AGT	rs5051	A	0.42	0.82
AGTR1	rs5186	C	0.25	0.05
CYP11B2	rs1799998	C	0.43	0.29
BDKRB2	n/a	Del	0.50	0.40

Table 3. Gene function and molecular and clinical phenotypes of candidate SNS genetic variants

SNS				
Gene	Gene function	Variant (common notation)	Molecular phenotype	Clinical phenotype
ADRB1	Mediates cardiac inotropy and chronotropy	Ser49Gly	Gly49 ↑ desensitization (8,9)	Gly49 ↑ survival (9)
		Arg389Gly	Arg389 ↑ function (10)	Arg389 ↓ survival (11)
ADRB2	Mediates cardiac inotropy and chronotropy	Gly16Arg	Gly16 ↑ desensitization (12,13)	Gly 16 ↑ survival (13)
		Gln27Glu	Glu27 desensitization resistant (12,13)	Gln27 ↓ risk of worsening HF (14)
ADRA2C	Pre-synaptic auto-inhibition of NE release	Codon 322-325 Ins/Del	Del ↓ function (15)	Del ↓ survival (16)
GRK5	Desensitization of beta-adrenergic receptors	Gln41Leu	Leu41 ↑ desensitization (17)	Leu41 ↑ survival (17)

Table 4. Gene function and molecular and clinical phenotypes of candidate RAAS genetic variants

RAAS				
Gene	Gene function	Variant (common notation)	Molecular phenotype	Clinical phenotype
ACE	Conversion of angiotensin I to angiotensin II	Intron 16 Ins/Del	Del ↑ plasma ACE (18)	Del ↓ survival (19)
AGT	Substrate for renin, converted into angiotensin I	G-6A	-6A ↑ transcription rate (4)	-6A ↓ survival (20)
AGTR1	Mediates major CV effects of angiotensin II	A1166C	1166C ↑ sensitivity (21)	1166C ↓ survival (22)
CYP11B2	Synthesizes aldosterone	T-344C	-344C ↑ plasma aldosterone (23-25)	-344C ↓ survival (24)
BDKRB2	Mediates CV actions of bradykinin	Exon 1 Ins/Del	Ins ↓ transcription rate (26,27)	Ins ↑ LV growth (27)

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