# THE ETIOLOGY OF LVH – A REVIEW OF GENETIC PREDISPOSITIONS

by

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# The Etiology of LVH – A Review of Genetic Predispositions

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My research fellowship was focused on assessing putative associations of polymorphisms in candidate genes with cardiovascular disease. Two previously well publicized associations were tested in the Dallas Heart Study (DHS), a stratified random population-sample of 6,101 Dallas County residents aged 18-65, with equivalent numbers of Black and non-Black women and men.[1] The first putative association tested was whether two polymorphisms in adrenergic receptors ($\alpha_{2C}$Del322-325 and $\beta_1$Arg389) synergistically increased the risk of heart failure in Blacks as reported by others (adjusted odds ratio, 10.1; 95% CI: 2.11 to 48.53; p=0.004).[2] In the DHS, we found that these variant alleles were not associated with self-reported heart failure or traits commonly accepted to be precursors for systolic HF, including left ventricular hypertrophy, increased left ventricular volume, reduced ejection fraction, and left ventricular mass (LVM).[3] The second putative association tested was whether polymorphisms in the $\alpha_{2A}$-adrenergic receptor (*Dra*I restriction fragment length protein) and the $\alpha_{2C}$-adrenergic receptor (Del 322-325) increased the risk of hypertension in Blacks.[4, 5] Again, we were

unable to replicate these findings. We found that these variant alleles were not associated with hypertension in Blacks in the DHS, alone or in combination.[6] Based on this experience, the present review was undertaken to analyze the genetic influence on a cardiovascular disease and determine whether lack of reproducibility of genetic association studies occurs commonly.

Given the breadth of this topic, I have chosen to focus on association studies of putative polymorphisms with the development of the "complex" trait of left ventricular hypertrophy (LVH). LVH can be defined as increased LV mass in relation to body size. The geometric pattern of this increased LV mass in LVH is important.[7] In response to pressure overload from conditions like hypertension, there is increased LV wall thickness leading to an increase in LV mass and the ratio of wall thickness to chamber dimension, the combination of which has been termed "concentric hypertrophy".[8] When volume overload conditions prevail, there is an increase in the left ventricular chamber volume with resultant chamber dilatation leading to a decrease in the ratio of wall thickness to chamber dimension, a pattern called "eccentric hypertrophy".[8] For purposes of this review, the focus will be largely on concentric hypertrophy. Although there are inherited monogenic causes of hypertrophy, ("familial hypertrophy cardiomyopathy"), established by linkage and family studies,[9] that body of work will not be evaluated in this review. Rather, the reproducibility of associations in polymorphisms with LVH performed in reported studies will be examined. Evidence looking at the role of polymorphisms in exercise-induced hypertrophy ("physiological" hypertrophy) also will be assessed. The review will conclude with a summary of "lessons learned" from previous work in this area with the intent of providing clues to improve future association studies in this field.

***LVH is a disease of clinical importance***

The development of LVH is common and occurs in 15-20% of hypertensive patients.[10] In one common paradigm, it is persistent hypertension that leads to the functional adaptation and development of LVH. However a number of insults can induce LVH, including overload, neurohormonal simulation, and oxidative stress.[11, 12] It is the compensatory hypertrophy that helps to maintain cardiac output as a reaction to physiologic and pathologic stimuli, including age, systolic blood pressure (BP), exercise, obesity, and other myocardial injury.[13]

The high prevalence of LVH is concerning since it conveys incremental risk beyond traditional major cardiovascular risk factors for adverse events including coronary artery disease, stroke, depressed ejection fraction and heart failure, arrhythmias, sudden cardiac death, and increased mortality .[14-19] Furthermore, the risk of increased LV mass has been shown to be continuous.[20, 21] Therefore, elucidating those factors which contribute to even small increases in LV mass will likely have clinical importance.

***Evidence to suggest that LVH has genetic underpinnings***

Animal studies have suggested that left ventricular mass may be influenced by genetic factors. In one model, LVH was observed in normotensive young spontaneously hypertensive rats.[22] In this model, LV mass was higher when compared to other rats despite similar levels of blood pressure.[23] Further, there was a range of LVH reduction in response to agents which lowered BP.[24, 25] Other data has suggested that a genetic locus in the spontaneously hypertensive rats affects LVM independently of blood pressure.[25]

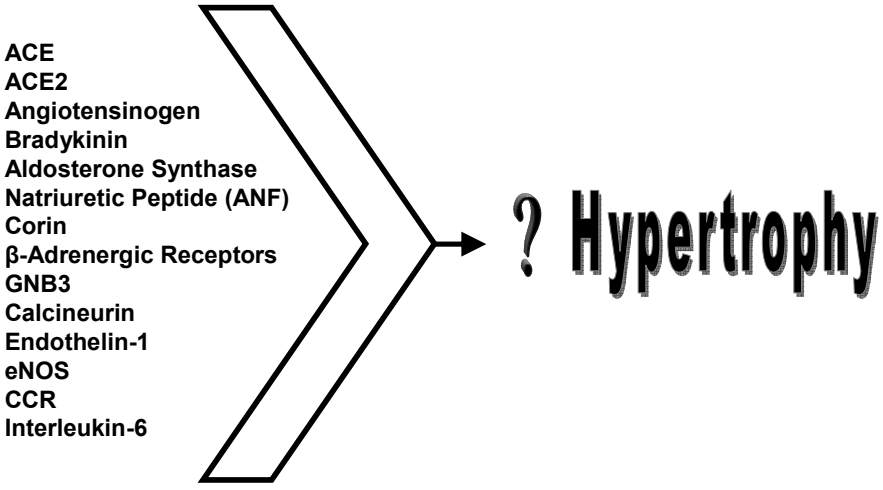
A variety of data from humans also supports the concept of underlying genetic predisposition towards LVH. First, there are considerable inter-individual differences in the development of increased LV mass for poorly understood reasons. Indeed, traditional risk factors including hypertension account for 25-50% of the variability in this trait.[26-28] Not all hypertensive subjects develop hypertrophy,[29] and the degree of LVH can vary significantly given a similar level of increased BP or even with normal blood pressure.[28] In fact, LVH can precede hypertension, suggesting again that some individuals are predisposed to the development of LVH likely secondary to genetic factors.[10]

There are important ethnic differences in the prevalence of LVH and certain segments of society appear to be particular susceptible. Specifically, considerable data has shown that American Blacks have a greater LVH frequency than Whites,[30-33] increased LVM,[28] and both increased LVM and wall thickness together,[33, 34] making heritable etiologies more plausible. Using the population-based sample in the DHS, after adjusting for gender, BP, and treatment along with subjects' socioeconomic differences, American Blacks have a 2-3 fold higher prevalence of LVH as compared to Whites, whether defined by height, BSA, or fat-free mass, and those ethnic differences persisted in multivariable models as well.[33] It would clearly be important to know if particular individuals in our population would be more susceptible to disease states and would possibly benefit from specific therapeutic measures. Recent data also has emerged which suggest that American Blacks have a predilection for hypertension due to an interaction of environmental exposures and genetic ancestral susceptilibility:[35] a similar paradigm for LVH is plausible. Twin and family studies also suggest that genetic factors may explain a portion of the variability of LV mass.[36-40] The heritability of LVM in Whites was

calculated in the Framingham Heart Study and Offspring study and shown to account for a small but distinguishable proportion of the overall variance in adjusted LVM, falling between .24 and .32.[41]

In total, the above data suggest that there is a genetic predisposition to the development of LVH. There has been considerable focus on important potential candidate susceptibility genes for LVH (*Figure 1*) and many association studies of these polymorphisms have been conducted, perhaps most frequently with polymorphism in the renin-angiotensin-aldosterone system, specifically in the angiotensin converting enzyme (ACE).[42, 43] For purposes of this review, these will be classified as being in the renin-angiotensin-aldosterone system (RAAS) or other pathways (non-RAAS). Although studies have been done analyzing how certain polymorphisms influence response to pharmacological therapy ("pharmacogenomics"), those data will not be covered here.

**FIGURE 1:** **Putative Candidate Genes for LVH**




Possible variant alleles that have been studied as predisposing genetic factors for LVH.

***Candidate Genes*** *(Table 1)*

A. RAAS

Angiotensin II is a potent hypertrophic stimulus.[44-47] Because angiotensin converting enzyme (ACE) is a critical enzyme in the production of angiotensin II, polymorphisms in ACE could affect angiotensin II levels and impact left ventricular mass. Recent data showing that ACE-inhibitors or angiotensin receptor blockers can lead to regression of LVH[48] support the concept that variation in these pathways may alter ventricular mass in humans.

There are several variants in RAAS that have been evaluated to date. These include the insertion/deletion polymorphism in the ACE gene; the homologue of the ACE gene, ACE2; in the angiotensinogen gene, the M235T polymorphism, G-6A polymorphism of its promoter region; the A1166C polymorphism of the AT1 receptor gene; the bradykinin 2 receptor (B2BKR) polymorphism; and the variant of the aldosterone synthase gene, CYP11B2.

a. ACE

The ACE gene has the presence or absence of a 287 bp fragment in a single *Alu* insertion/deletion (I/D) polymorphism in intron 16, which is associated with ACE levels in plasma. In subjects with one or two D alleles, 25-50% higher ACE levels were observed both in plasma,[49, 50] and at tissue sites[51] when compared to the I/I genotype,[52] though evidence demonstrating that these higher levels are from an increased conversion of angiotensin I to angiotensin II is scant.[53] Nevertheless, many association studies have been conducted using this variant allele. In one pioneering study on the ACE allele, the homozygous D-allele (D/D) was significantly associated with LVH in men but not

women using multivariable conditional logistic regression with adjustment for possible confounding variables.[54] That study used a population-based sample including 141 women and 149 men with evidence of LVH; however ECG rather than the more accurate modalities of echocardiography or MRI, were used to define LVH. Many other studies have assessed whether the insertion/deletion polymorphism of the ACE gene is associated with LVH with conflicting results.[54-63] Of note, the ACE genotype, the most widely accepted candidate locus for LVH risk, was not associated with LVM or LVH in the Framingham Heart Study after adjusting for covariates, using sophisticated measures such as genetic-linkage algorithms.[58] In 1,919 Japanese subjects, one multicenter study found that for women with EH, the D/D genotype was associated with thickness of interventricular septum, but not in men.[55]

In 2000, a meta-analysis was conducted of the 28 case-control and cross-sectional studies (n=6638) which evaluated the putative association of the ACE I/D and LVH in humans, mainly in Whites.[60] Compared with the I/I genotype, the risk of LVH associated with the D/D was 14% higher (p=0.23) and with D/I alleles was 5% higher (p=0.61); however, in a sensitivity analysis, the D/D genotype was associated with a significantly higher risk of LVH when compared with I/I, in untreated hypertensive subjects only (p=0.002).[56, 60, 64, 65] The odds of LVH associated with the D-allele ranged from 0.76-2.33 [65, 66] in the analyzed studies, achieving significance in only three.[54, 56, 65] When combined, the overall association with LVH was not significant (odds ratio 1.09 (95% CI: 0.98-1.21), p=0.12). LVM was also analyzed as a continuous trait, and no association was present with the D/I allele, though in the untreated hypertensive subjects, LVM was significantly higher in those with D/D versus those with the I/I genotype (p=0.001).

There was no reported evidence of publication bias after funnel plot analysis. The investigators noted that a great deal of heterogeneity was present in the studies including varying definitions of LVH[60] and end points. Racial variation in allele frequency may make the ACE polymorphism more useful in some groups than others.[67]

b. ACE2

A homologue of the ACE gene on the X-chromosome, termed ACE2 was another candidate gene assessed for an association with LVH. ACE2 is expressed in the heart[68] and it is thought that ACE2 may degrade angiotensin II and produce vasodilator activity.[69] It has been found to be a candidate gene for hypertension in rat models, and disruption of the gene resulted in impaired cardiac function.[70] Four single nucleotide polymorphisms (SNPs) (rs4646156, rs879922, rs4240157, and rs233575) in linkage disequilibrium (LD) define a TGGC haplotype. In a substudy of the MONICA survey, the minor alleles of the four ACE2 SNPs were found to be associated with prevalent LVH and higher LVM and septal wall thickness in men.[71]

c. Angiotensinogen

Angiotensinogen is cleaved by renin to form angiotensin I. SNPs in angiotensinogen have also tested as risk factors for LVH. The methionine/threonine (M235T) variant is located on exon 2 of the angiotensinogen gene, and has been linked to HTN.[72-74] The G-6A variant is an angiotensinogen variant that forms from a guanine to adenine substitution, and is in linkage disequilibrium with M235T.[75-77] The M235T and G-6A polymorphisms were weakly associated with LVM independent of covariates in hypertensive patients.[77] Similarly, in another study with 175 Spanish subjects with

essential hypertension (EH) from an outpatient clinic, neither the M235T nor T174M variants were associated either with hypertension or with electrocardiographic LVH.[78]

d. Combinations of SNPs

Multiple variant alleles were evaluated in 156 subjects from another general population cohort, including the polymorphisms of the ACE gene, the A1166C polymorphism of the ATI receptor gene, the M235T polymorphism, and the 6-G/A polymorphism. LVM was assessed by echocardiography.[79] There was no association of LV mass with any of the tested variants, alone or in combination.[79] The European Project on Genes in Hypertension (EPOGH) study analyzed the ACE and the aldosterone synthase variant in 219 families (382 parents and 436 offspring), and a relationship was found between LVMI and the ACE gene (D/I) in Slavic and Italian populations, but no association was found between LVM and -344C/T polymorphism.[80]

e. Bradykinin

Given that cardiac growth is thought to be mediated in part via kinin modulation, polymorphisms in the bradykinin 2 receptor (B2BKR) have also been implicated as putative risk factors for LVH. Histological and structural evaluation in knockout mice for the B2BKR gene demonstrated ventricular hypertrophy and cardiomyocyte enlargement.[81] Of three B2BKR polymorphisms, the 58T/C on the promoter region has been associated with hypertension.[82] In Japanese subjects, the 58T/C polymorphism was associated with LVH among hypertensives. In addition, LV mass was significantly higher in the subjects with both the B2BKR C/C genotype and the ACE D allele as compared to those with other genotypes, again suggesting a synergistic effect.[83]

f. Aldosterone synthase

In the adrenal cortex, aldosterone is produced by a mitochondrial cytochrome P450 enzyme, aldosterone synthase.[84] The CYP11B2 -344C/T polymorphism lies in the promoter region of the aldosterone synthase gene and the T-allele is associated with increased levels of aldosterone production.[85] Given the reduction in morbidity and mortality from aldosterone antagonists,[86] certain genetic targets have been implicated to alter hypertrophic response. Again, there have been conflicting results in the literature as to whether this SNP associates with LVH and LVM; several studies have shown a positive association,[87-90] and others found no association.[80, 91, 92] A more recent study focused on highly homogeneous White middle-aged subjects with mild to moderate HTN recently diagnosed or never treated, and demonstrated a positive relationship between the polymorphism and LV mass, LV septal thickness and relative wall thickness, with an additive affect of the T-allele in hypertensive subjects.[88] This contrasts a study with 84 subjects, demonstrating that those with the C/C genotype had greater LVM, albeit in normotensive subjects.[90] The association is independent of adrenal aldosterone production and the authors proposed a role for cardiac aldosterone production.[88]

g.Other genes (non-RAAS)

Associations with LVH have been assessed with other genetic variants: an insertion/deletion polymorphism identified on the 5'-flanking region of the type A human natriuretic peptide (NP) receptor gene; the human atrial natriuretic factor (ANF) gene; the genetic variant in the GNB3 gene, C825T; the corin gene; $\beta_2$ adrenergic receptor genes; a novel 5-base pair deletion in calcineurin B promoter region; endothelin-1 gene, and an eNOS allelic variant involved in regulation of BP. A newer focus has been on the role of

inflammation and associations of polymorphisms in the chemokine receptors (CCR); and the interleukin (IL)-6 promoter, and their relationship with LV mass.

i. Natriuretic Peptides

The natriuretic peptide (NP) family is involved in the regulation of BP and extracellular fluid volume status.[93] In a mouse model, targeted deletion of the type A NP receptor resulted in hypertension, LVH and sudden death.[94] This deletion may influence cardiac remodeling and BP regulation. In one human study, performed in Japanese subjects, an insertion/deletion (I/D) polymorphism was identified on the 5'-flanking region of the type A human NP receptor gene (NPRA).[95] Out of 200 subjects with essential hypertension, 8 subjects had a heterozygous deletion for this allele and 4 of these were found to have LVH. In 200 normotensive control individuals, 1 had the heterozygous deletion and also had evidence of LVH. The variant allele was also associated with significantly elevated B-type natriuretic peptide (BNP) but not atrial natriuretic protein (ANP) plasma levels in individuals with essential hypertension.[95]

The atrial natriuretic factor (ANF) has dominant vasodilatory and natriuretic effects in addition to inhibiting formation and release of aldosterone and renin activity.[96] Genotypes for the insertion/deletion (I/D) polymorphism are located in the ANF gene on the second intron. Previously, the ANF I/D polymorphism was reported to not be associated with hypertension in the Gulf Arab population.[97] However, in a similar population, more recent data demonstrates significant differences in LVH between subjects based on their ANF, D allele.[98]

ii. Corin

Another promising genetic link to LVH is the corin gene. Corin knockout -/- mice have spontaneous hypertension when compared with wild type mice, a difference which is enhanced by dietary salt loading. In addition, the corin -/- mice exhibit cardiac hypertrophy.[99] In humans, the corin T55I/Q568P allele has been found to be enriched in Blacks and to be associated with elevated BP.[100] Further, the T555I/Q568P locus was shown to be a risk factor for increased LV mass in response to elevated blood pressure, both in the Dallas Heart Study (DHS) and the Multi-Ethnic Study of Atherosclerosis (MESA).[101]

iii. β Adrenergic Receptors

The importance of sympathetic activity with catecholamines in worsening decline and cardiac failure is well established.[102, 103] The catecholamines act upon the heart by binding to adrenergic receptors (AR). Both $\beta_1$- and $\beta_2$-AR subtypes in the heart couple to Gs-proteins to activate adenylyl cyclase, and increase the intracellular level of cAMP.[104]

The $\beta_2$-ARs are present in 1) myocardium where they augment heart rate and contractility and 2) in the vasculature, where their effects alter peripheral vasodilatation.[104] Four $\beta_2$-ARs SNPs: $\beta_2$Arg16Gly, $\beta_2$,Gln27Glu, and $\beta_2$ Thr164Ile, and a promoter region variant (-47C/T) have all been implicated in decreased cardiac function, and hypertension.[105-108] Using a combined linkage association study in normotensive twin subjects, predominantly $\beta_2$Arg16Gly, was functionally associated with BP and increased cardiac dimensions.[109] Another association study was performed on 775 hypertensive individuals to assess the $\beta_2$ genotype and LVM. There was a significant

association found between the $\beta_2$Gln27Glu variant and indexed LVM, which stayed positive after corrections for confounding factors in the model.[110]

The $\beta_1$-AR is a key regulator of cardiac function and is the predominant cardiac subtype.[104] The $\beta_1$-AR gene is on the long arm of chromosome 10. In mouse models, overexpression of this receptor lead to chronic sympathetic activity and cardiac decline.[111] Several SNPs have been described, and the gain-of-function polymorphism $\beta_1$Arg389Gly has enhanced receptor-$G_s$ interaction with the increased activation of adenylyl cyclase.[112] In one study of 249 white subjects with renal disease, echocardiography demonstrated a significant increase in LVM in subjects with both the CC and CG genotypes, when compared to the wild type.[113]

iv. GNB3

The GNB3 gene encodes for the G-protein $\beta_3$ subunit and is thought to play a role in the $Na^+$-$H^+$ exchanger activity, which in turn regulates cell volume and blood pressure.[114] There is a single base pair C825T substitution in the GNB3 variant gene located in exon 10. It has been reported that in subjects with essential hypertension, there is a significant association of the T allele in a variant of the GNB3 gene thought to be from the underlying mechanism of augmented $Na^+$-$H^+$ activity,[115] but there are conflicting data as to whether this variant is associated with elevated BP.[115-117] When assessing the relationship of this variant to LV parameters, it was found that the combination of the genotypes, (CT and TT) had significantly higher left ventricular end-diastolic diameter, posterior wall thickness, and indexed LVM as measured by echocardiography. In addition, the combined group of heterozygotes and homozygotes, had significantly more LVH when compared to the wild type. In logistic regression

models, LVH was the only variable independently associated with the T- allele.[118] However, there was no association found with the T-allele in 211 Gulf Arabs, but instead the C/C was significantly associated with LVH.[119]

v. Calcineurin

Calcineurin is a calcium/calmodulin-dependant protein phosphatase that plays a key role in moderating the hypertrophic response of the heart in animal models.[120] It contains a catalytic subunit, calcineurin A, and a regulatory subunit bound to $Ca^{2+}$, calcineurin B.[120, 121] The activation of calcineurin occurs through the binding of calcium/calmodulin with sustained levels of $Ca^{2+}$ concentration, which can occur when the heart is under stress or neurohormonal stimulation.[120, 122-124] In animal experiments, transgenic mice with overexpression of active calcineurin or nuclear factor of activated T-cells 3 (NFAT)3 develop cardiac hypertrophy.[125] Calcineurin activity has been reported to be increased in hypertensive-related LVH.[126] A novel 5-base pair insertion/deletion polymorphism in the calcineurin B gene, PPP3R1, was evaluated in one human study for its association with LVH and increased LVM. In hypertensive Blacks alone, or in Blacks and Whites combined, the heterozygous and homozygous carriers of the 5D allele had increased LVM, as assessed by echocardiography, compared to the remainder of the cohort.[124] This association has not been validated to our knowledge.

vi. Endothelin-1

Endothelin-1 (ET-1) is peptide produced by vascular endothelial cells. It increases stress-induced sympathetic activity and is a potent arterial vasoconstrictor, that regulates body fluid volume.[127, 128] Animal studies demonstrated that BP regulation is

altered in knockout mice models for the ET-1 gene.[129] In addition, in hypertensive rats, cardiac ET-1 influenced and contributed to LVH.[130] Plasma ET-1 levels were found to be elevated in human subjects with hypertension.[131] The severity of LVH correlated with the level of ET-1 in plasma,[132] making ET1 a putative candidate gene for increasing risk of LVH. Four SNPs in the ET-1 gene (T-1370G, +138/ex1 D/I, T-37/in2C, Lys198Asn) and were tested as risk factors for hypertension and LVM. In the 537 younger subjects, LVM levels were higher in carriers of the -1370G allele, only in the lower socioeconomic group, which was confirmed with haplotype analysis.[128] The remaining 3 SNPs had no significant effect on LVM.

vii. eNOS

Nitric oxide (NO) is synthesized in the vascular endothelium by endothelial nitric oxide synthase (eNOS) and is involved with the physiologic regulation of BP and remodeling.[133] In animal studies, eNOS knockout mice developed increased BP, increased wall thickness and LVM, [134, 135] and deletion of NOS (NOS3-/-, NOS1-/-), leads to systemic hypertension, LVH, and increased wall thickness.[136] The eNOS gene, contains 26 exons and is located on chromosome 7. Several polymorphisms have been identified in humans, but there are limited data testing their association with LVH. The Glu298Asp (G894T) polymorphism at exon 7 of the NOS3 gene was examined for its impact on longitudinal development of BP and LVM in youths, along with -922A>G and variable tandem repeats (VNTR) at intron 4.[137] Age and gender-dependent effects on diastolic BP were found for HTN risk, but no associations with the polymorphisms or haplotypes for systolic BP or LVM were found in 579 subjects.[137] In one population-based study from OPERA, 600 hypertensives and 600 controls were evaluated and

demonstrated only a 0.07mm difference in the intima-medial thickness and indexed LVM by echocardiogram, and no significant associations were found between eNOS Glu298Asp polymorphism and BP levels, LVM or LVH.[138] However in a Russian study, there was an association between the Glu allele (homozygotes and heterozygotes) of NOS3 with LVH in 109 subjects.[139]

viii. Inflammatory pathways

Some newer studies have focused on inflammation and its role in the pathogenesis of LVH. The rationale for this association is the relationship proposed between inflammatory mediators and inflammatory-coupled vascular destruction and LVH.[140, 141] Putative candidates in this pathway included the chemokine receptors (CCR) and the interleukin (IL)-6 promotor polymorphism. In mice the inflammatory cytokine cardiotropin 1, has been shown to induce hypertrophy in cardiomyocytes.[141] In patients on hemodialysis, the interleukin (IL)-6 promotor polymorphism 174G/C has been associated with elevated BP and LVH.[140] Individuals with the GC or CC genotype were found to have a significantly higher diastolic blood pressure and LVMI than GG homozygotes.[140] A relationship of essential hypertension and the polymorphisms of the CCR genes, CCR5Δ32 and CCR264I, have been reported, and a possible association with LVH was suggested.[142] However, a subsequent analysis from this same group demonstrated no association of these polymorphisms with LVH in hypertensive subjects. These disparate results may suggest unique genetic risk factors for hypertension and LVH.[143]

ix. Summary of candidate genes and LVH (*Table 1*)

It is clear that to date there has been substantial problems with reproducing association studies of candidate polymorphisms and LVH. Potential causes for this will be discussed later in this review.

***Genetic contribution to variation in exercise-induced ("physiological") hypertrophy***

Identification of genetic risk factors may be enhanced by assessing inter-individual variation in response to the same stress. Exercise-induced ventricular hypertrophy is one such model that has been utilized to identify genetic risk factors for LVH.

Exercise-induced hypertrophy is distinct from maladaptive pathologic hypertrophy in several ways. First, in exercise-induced hypetrophy, contractility is preserved along with ATPase activity.[144-146] Second, pathological hypertrophy is characterized by an abnormal accumulation of collagen which is not seen in physiological hypertrophy.[147] Perhaps related to this, myocardial perfusion (MP) assessed by PET scanning demonstrated higher perfusion reserve in athletes with physiological hypertrophy as compared to normal controls, whereas patients with pathological (hypertensive) LVH had lower perfusion reserve versus controls.[148] Third, although trained athletes and hypertensive patients had comparable increases in LV mass, the former (physiological hypetrophy) was not accompanied by impairment in LV filling which was seen with pathological hypertrophy.[149] Fourth, exercise induced hypertrophy was associated with a different gene expression than seen in pathologic (hypertensive) hypetrophy.[150] Finally, physiologic hypertrophy is not generally deleterious in the 3

major settings where it has been identified, (including exercise, maturation in infancy, and during pregnancy),[8] possibly due to poorly characterized differences in activated signal pathways compared to other states of pathological hypetrophy.

The renin-angiotensin-aldosterone system (RAAS) may be involved in the cardiac growth of athletes. As with pathological hypertrophy, the angiotensin converting enzyme (ACE) Insertion/Deletion (I/D) has perhaps been the most studied candidate polymorphism in this regards (*Table 2*). Physical exercise stimulates the RAAS,[151] which may in turn regulate cardiac growth.[152] However not all endurance athletes develop similar LV mass enhancement, suggesting genetic factors may play a role.[153] As described above, the deletion (D/D), rather than the insertion, is associated with higher circulating ACE levels,[49, 154] and is associated with elevated ACE levels in the human myocardium.[51] In one study looking at the frequency of the ACE genotype among athletes, there was a significant enrichment of the I (insertion) allele along with a reduction of the D (deletion) allele in endurance athletes when compared to controls.[155] Because the I allele is associated with decreased ACE level, it may lead to less pathological ventricular hypertrophy possibly enhancing cardiac performance. In contrast, the ACE polymorphism was compared in 56 endurance athletes and 46 sedentary subjects using echocardiographic evaluation, and indexed LVM was significantly higher in athletes with the D-allele than in controls (P= <0.001).[156] Another study evaluated a group of 61 White athletes, and compared ACE genotypes. The D/D variant was again associated with a higher LVM than the I/D regardless of other cofounders.[157] No association was found in endurance athletes looking at ACE, M235T,

and A1166C polymorphisms separately, but in combination analysis, those with ACE D/D, and angiotensinogin T/T genotypes had greater LVM.[158]

With training of athletes, there appears to be more consistency in the results of the association studies, though the number of such studies remains modest (*Table 3*). At the end of a 10-week physical training period in White male military recruits, wall thickness and LVM increased in proportion to the number of D-alleles.[159] Further, the prevalence of ECG defined LVH increased significantly only among those with the D/D variant.[159] Exercise-induced increases in LVM, were similar in athlete-soccer players with the D/D and D/I allele, and were significantly higher than subjects with the I/I genotype after the training period.[160] There were no differences in the frequency of ACE genotypes between the cases (athletes) and controls prior to training in this study,[160] demonstrating that changes of a trait in response to an imposed stress may be a more powerful study design than a cross-sectional association study.

Of the B2BKR polymorphisms the +9/-9 I/D in exon 1 was associated with left ventricular physiologic growth in normotensive men undergoing physical endurance training.[161] This deletion polymorphism is associated with lower concentrations of bradykinin and a greater degree of LVH.[162] In 109 male subjects tested, physical training increased LV mass measured by cardiac MRI to a greater degree in those homozygous for the B2BKR variant allele alone, and with both the B2BKR and ACE deletion (D/D) as compared to the rest of the cohort.[162] There appeared to be a synergistic effect between these two alleles.

Polymorphisms in the peroxisome proliferator-activated receptor α (PPARα) have also been implicated as mediators of physiologic cardiac hypertrophy. The PPARα gene

is involved with uptake and oxidation of fatty acids in cardiomyocytes, resulting in cardiac growth.[163] In a study involving 144 subjects, the C/C and C/G variants located in intron 7 of the gene, significantly affected growth in response to physical exercise, and the greatest increase in LVM was found in subjects with the C/C variant of the PPARα gene and the D/D variant of the ACE gene.[164]

Genetic factors appear to be associated with degree of physiological hypertrophy induced by a training period, but not consistently when comparing baseline LV mass in athletes to controls. The improved reproducibility of association studies using change in LV mass induced by endurance training as the outcome variable suggests that measuring a response to an imposed stress may be a more effective study design.

***Lessons from Past Studies and Future Direction***

The discovery of a genetic marker that could be used to predict increased or decreased risk of development of LVH would have significant implications for risk stratification. Many of the reviewed candidate alleles (*Figure 1*) may indeed play a role in the development of LVH, but given the conflicting data (*Table 1*), one must be cautious in accepting these associations. Studies of even the most widely investigated variant for LVH, the ACE I/D allele, had poorly reproducible results. This is not surprising given that of 166 potential associations for common allelic variants and reported diseases which were analyzed three or more times, only 6 had been consistently replicated in the literature.[165] There are likely several important factors explaining the lack of reproducibility of previous association studies (*Table 4*).

**TABLE 4: Contributing Factors to Poor Reproducibility of Present Association Studies**

| |
|---|
| **1. False positive associations** |
| **A. Multiple testing** |
| **i. Number of genes** |
| **ii. Interaction with environment** |
| **B. Inappropriately high P values** |
| **C. Hidden population stratification** |
| **D. Heterogeneity** |
| **E. Publication Bias** |
| **2. Assessment of non-functional polymorphisms** |
| **3. Lack of validation prior to initial publication** |
| **4. Underpowered studies** |

False-positive results can be due to the inappropriately high p-values that may be used when multiple associations are being assessed and contribute to type I error. For example, if 1000 polymorphisms are being evaluated separately with a p-value threshold set at $< 0.05$ for significance, then 5% or 200 distinct associations will be found solely due to chance alone. Declaring success with a large number of analyses therefore must be done with lower, more stringent p-values, and appropriately conservative statistical corrections, like the Bonferoni correction or Sidak correction. The critical decision regarding the setting of the nominal p value for "significance" is demonstrated by data which show that two studies with $p < 0.01$ or a single study other than the first positive one with a $p < 0.001$, is strongly predictive of a subsequent reproducible study.[166] Proper

evaluation should also take into account the multiple tested hypotheses and prior probability using a Bayesian approach.[167]

Potential problems in association studies due to multiple comparisons is magnified when one considers analyzing hundreds or thousands of single nucleotide polymorphisms (SNPs), and further still when considering gene-environment interactions[168] involving multiple genes and exposures. The sheer number of SNPs,[169] some of which may be sequencing errors or rare variants that are population specific,[170, 171] and many of which do not alter coding sequence and are not likely to be functional,[171, 172] make selection of candidate polymorphisms challenging.[173] The influences of environment need to be accounted for as well in analysis. For example, lower socioeconomic status (SES) was an independent predictor of increased LVM among hypertensive and normotensive Blacks,[174] possibly due to increased physiologic stress, cardiovascular reactivity and hypertension.[175-177] There are other potential contributors to false positive associations including unaccounted for differences in population stratification, when cohorts with different demographics and rates of disease are combined, allowing a disproportionate representation of traits.[167] Heterogeneity in results between populations of two or more studies can occur if unique environmental or genetic differences influence the variant allele's contribution to a particular trait, or it can also occur if the tested variant is not truly causal, but instead is in linkage disequilibrium with the causal variant.[167] Yet another contributor is publication bias, whereby positive significant studies are more likely to be published than negative ones, which either are not submitted, or are rejected from journals.

To guard against the possibility of false positive association due to the many factors elucidated above, validation of associations in additional cohorts is critical. Having an association validated in additional cohorts prior to publication would seem to be a necessary standard. Indeed, this has been the approach taken by some major genetic journals (e.g., Nature Genetics) in the absence of compelling in-vitro functional data.

Power is another major concern with genetic studies and the reduction of type II errors needs to be addressed.[173] Adequate sample size is critical, to defend against false-negative associations from underpowered studies,[166] especially if there is only a modest effect size of a common variants.[166, 167] Some cases where initial observations cannot be validated may in fact be due to the "validation" cohort being underpowered.

***Haplotypes, Admixture, and QTL: Advancing Association Studies?***

Genetic investigation has progressed over the years, from single-marker gene studies to the development of polymerase chain reaction (PCR) and genome-wide linkage analysis. With these technological advances in gene cloning and sequencing, and completion of the human genome project, large numbers of markers and SNPs now can be analyzed in any given cohort with rapid high-throughput methods.[167] Genotyping of cohorts for multiple SNPs has allowed alternative analytic approaches. Linkage disequilibrium (LD) models are commonly used and allow indirect association mapping.[171] This is enhanced with haplotypes, which are based on the assembly of closely linked alleles inherited together on the same chromosome.[171] It is thought that haplotype analysis may prove more powerful than association studies with single SNPs, since one haplotype encompasses a span of DNA which includes numerous SNPs. In

essence, such an approach would provide an effective way to reduce the number of putative risk factors to be assessed. Another approach to improve association studies is admixture mapping. Here, information can be generated about candidate genes from the admixture of ancestrally distinct populations (eg, degree of African ancestry). If marker alleles differ in frequency between the two populations (Whites and Blacks, eg), and the markers are in linkage disequilibrium with a causal variant, then the degree of admixture can be used to map disease-associated genes[178-180] via a genome scan. Quantitative trait loci (QTLs) are another approach advocated to study genetic risk factors of complex traits. It is the study of quantitative genetic traits (biometrical) as opposed to traditional Mendelian (discrete) characteristics, in that there is not a one-to-one relationship between phenotype and genotype.[181, 182] However, very few of the QTLs discovered to date have led to the identification of candidate genes.[181]

***Conclusions***

There are reasonable data to suggest that there are important genetic influences in the development of LVH including animal data, measures of hereditary, ethnic differences, and unexplained inter-individual variations in development of pathological hypertrophy in response to hypertension and physiological hypertrophy in response to exercise. Nevertheless, dissecting these underlying genetic causes of complex traits remains a challenge[182] for multiple reasons including the vast array and diversity of potential candidate polymorphisms, the likely modest effect of a particular variant on a phenotype, potential important interactions with environment, and the heterogeneity of study cohorts and populations.[60, 181] Given the high false positive rate and the

underpowered negative analyses found with genetic association studies in the literature, improved statistical methodology coupled with large derivation and validation cohorts are needed.[183] Association studies based on assessing changes of a trait in response to an imposed stress also likely will be helpful based on our review of the literature (*Table 3*).

Our inability to validate two putative associations in the Dallas Heart Study[3, 6] is not unexpected given similar experience with other attempts to validate reported associations. Indeed, confirmed genetic risk factors for LVH are scant, but we are hopeful that insights into the heritable etiology of this disease state may soon be exposed by a growing interest in this field coupled with enhancements in study design.

## Abbreviation Dictionary:

| | | |
|---|---|---|
| Adrenergic Receptors | = | (AR) |
| Angiotensin Converting Enzyme | = | (ACE) |
| Atrial Natriuretic Factor | = | (ANF) |
| Blood Pressure | = | (BP) |
| Body Surface Area | = | (BSA) |
| Bradykinin 2 Receptor | = | (B2BKR) |
| Brain Natriuretic Peptide | = | (BNP) |
| Chemokine Receptors | = | (CCR) |
| Dallas Heart Study | = | (DHS) |
| Electrocardiograms | = | (ECG) |
| Endothelin-1 | = | (ET-1) |
| Endothelial Nitric Oxide Synthase | = | (eNOS) |
| Essential Hypertension | = | (EH) |
| Hypertension | = | (HTN) |
| Insertion/Deletion | = | (I/D) |
| Interleukin | = | (IL) |
| Linkage Disequilibrium | = | (LD) |

| | | |
|---|---|---|
| Left Ventricular Hypertrophy | = | (LVH) |
| Left Ventricular Mass | = | (LVM) |
| Magnetic Resonance Imaging | = | (MRI) |
| Multi-Ethnic Study of Atherosclerosis | = | (MESA) |
| Myocardial Perfusion | = | (MP) |
| Natriuretic Peptide | = | (NP) |
| Nitric Oxide | = | (NO) |
| NP receptor gene type A | = | (NPRA) |
| Nuclear Factor of Activated T-cells | = | (NFAT) |
| Peroxisome Proliferator-Activated Receptor α | = | (PPARα) |
| Polymerase Chain Reaction | = | (PCR) |
| Protein Kinase C | = | (PKC) |
| Renin-Angiotensin-Aldosterone System | = | (RAAS) |
| Quantitative Trait Loci | = | (QTLs) |
| Single Nucleotide Polymorphisms | = | (SNPs) |
| Socioeconomic Status | = | (SES) |
| Variable Tandem Repeats | = | (VNTR) |

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## TABLE 1. Association Studies of Candidate Genes and Left Ventricular Hypertrophy (LVH)

| Alleles | Study | Sample Sizes | Result of Study (+/-) | Odds Ratio (95% CI) | P-value |
|---|---|---|---|---|---|
| RAAS | | | | | |
| ACE | | | | | |
| | ‡ Kuznetsova et al. (2000)[60] | 4094 (12 studies) | (-) for D allele | 1.09 (0.98-1.21) | P=0.12 |
| | † Schunkert et al. (1994)[54] | 580 | (+) for D/D | 1.74 (1.22-2.49) | P= 0.002 |
| | | | (-) for D allele | 1.00 (0.65-1.55) | P=1.00 |
| | † Kupari et al. (1994)[62] | 86 | (-) for D/D | 0.68 (0.18-2.57) | P=0.57 |
| | | | (-) for D allele | 0.85 (0.21-3.49) | P=0.82 |
| | † Lindpaintner et al. (1996)[58] | 2439 | (-) for D/D | 1.12 (0.88-1.43) | P=0.35 |
| | | | (-) for D allele | 0.90 (0.68-1.19) | P=0.47 |
| | † Gharavi et al. (1996)[64] | 64 | (-) for D/D | 0.63 (0.23-1.75) | P=0.38 |
| | | | (-) for D allele | 1.36 (0.36-5.08) | P=0.65 |
| | Saeed et al. (2005)[63] | 180 | (+) for D/D | 0.32 (0.16-0.66) | P=0.001 |
| | | | (-) for D allele | 1.03 (0.26-4.04) | P=0.97 |
| ACE2 | | | | | |
| | Lieb et al. (2005)[71] | 160 | (+) TGGC Haplotype | 3.10 (1.38-6.96) | P=0.006 |
| BDKRB2 | | | | | |
| | Fu et al. (2004)[83] | 716 | (+) for T/C | 3.9 (1.0-3.3) | P=.048 |
| CYP11B2 | | | | | |
| | Schunkert et al. (1999)[92] | 1234 | (-) for T- alleles | 1.16 (0.58-2.34) | P=0.67 |
| PPP3R1 | | | | | |
| | Tang et al. (2005)[124] | 368 | (-) 5D for LVH (+) LVM | 1.23 (0.66-2.28) | P=0.51 |
| CCR | | | | | |
| | Mettimano et al. (2005)[142] | 118 | (-) CCR5Δ32 | 0.61 (0.05-6.87) | P=0.68 |
| | | | (-) CCR264I | 0.90 (0.35-2.34) | P=0.83 |
| eNOS | | | | | |
| | Karvonen et al. (2002)[138] | 1024 | (-) for Glu298Asp in all subjects | 0.90 (0.49-1.67) | P=0.74 |
| GNB3 (GN3B) | | | | | |
| | Obineche et al. (2001)[119] | 211 | (-) for for T-allele | 0.48 (0.27-0.86) | P=0.013 |
| | Poch et al. (2000)[118] | 86 | (+) for T-allele | 3.78 (1.43-9.97) | P=0.006 |

* Data is Unadjusted for homozygotes, heterozygotes, and wild type analysis for LVH
‡ Meta Analysis
† Larger Studies included in meta-analysis

**TABLE 2. Association of ACE Insertion-Deletion and LV mass ("Physiological Hypertrophy")**

| Study | Sample Size | LVMI by Genotypes D/D | D/I | I/I | P-value |
|---|---|---|---|---|---|
| Diet et al. (2001)[158] | 83 | 155.3 ± 31.3 | 145.3 ± 27.8 | 140.9 ± 24.0 | P=0.247 |
| Hernandez et al. (2003)[157] | 61 | 162.2 ± 36.5 | 141.6 ± 34 | 127.7 ± 17.5 | P=0.031 |
| Tanriverdi et al. (2005)[156] | 56 | 181.5 ± 44.1 | 121.4 ± 28.3 | 106.3 ± 15.8 | P=0.001 |

Values are means ± SD
$g/m^2$ used for indexing

**TABLE 3. Association of ACE Insertion-Deletion and Change in LV mass ("Physiological Hypertrophy") with Training**

| Study | Genotype | Sample Size | Pre-Training | Post-Training | P-value |
|---|---|---|---|---|---|
| ‡ Montgomery et al. (1997)[159] | | 280 | | | |
| | D/D | | 34.7 | 43.6 | P=0.0001 |
| | D/I | | 36.2 | 44.7 | P=0.0001 |
| | I/I | | 37.7 | 37.9 | P=0.81 |
| † Fatini et al. (2000)[160] | | 56 | | | |
| | D/D + D/I | | 109.8 ± 18.7 | 123.8 ± 22.6 | P=0.005 |
| | I/I | | 107.8 ± 5.1 | 104.2 ± 15.2 | P=0.71 |

Values are means or means ± SD
‡ $g/m^{2.7}$ used for indexing
† $g/m^2$ used for indexing