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ORIGINAL INVESTIGATION

RAAS gene polymorphisms influence progression of pediatric hypertrophic cardiomyopathy

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Abstract Hypertrophic Cardiomyopathy (HCM) is a disease with variable rate of progression. Young age is an independent risk factor for poor outcome in HCM. The influence of renin–angiotensin–aldosterone (RAAS) genotype on the progression of HCM in children is unknown. Children with HCM ($n = 65$) were enrolled prospectively across two centers (2001–2005). All subjects were genotyped for five RAAS gene polymorphisms previously associated with LV hypertrophy (pro-LVH): *AGT M235T, ACE DD, CMA*-1903 *A/G, AGTR1* 1666 *A/C and CYP11B2*-344 *C/T*. Linear regression models, based on maximum likelihood estimates, were created to assess the independent effect of RAAS genotype on LV hypertrophy (LVH). Forty-six subjects were homozygous for <2 and 19 were homozygous for ≥2 pro-LVH RAAS polymorphisms. Mean age at presentation was 9.6 ± 6 years. Forty children had follow-up echocardiograms after a median of 1.5 years. Indexed LV mass (LVMI) and LV mass z-scores were higher at presentation and follow-up in subjects with ≥2 pro-LVH genotypes compared to those with <2 ($P < 0.05$). Subjects with ≥2 pro-LVH genotypes also demonstrated a greater increase in septal thickness (IVST) and in LV outflow tract (LVOT) obstruction on follow-up ($P < 0.05$). On multivariate analysis, a higher number of pro-LVH genotypes was associated with a larger effect size ($P < 0.05$). Pro-LVH RAAS gene polymorphisms are associated with progressive septal hypertrophy and LVOT obstruction in children with HCM. Identification of RAAS modifier genes may help to risk-stratify patients with HCM.

Introduction

Hypertrophic Cardiomyopathy (HCM) is a myocardial disease predominantly of genetic etiology characterized by non-physiologic left ventricular hypertrophy (LVH), diastolic dysfunction, arrhythmias, and sudden death. It remains the leading cause of sudden death in children and young adults in the US (Maron et al. 1995). The etiology is multi-factorial with majority of cases occurring secondary to mutations in genes encoding the sarcomere proteins. Several 100 mutations in over a dozen sarcomere myofilament genes have been identified which contribute to the heterogeneity of the disease phenotype (Tardif 2005). However, even among family members carrying the same disease mutation, variability of cardiac phenotype and clinical course occurs frequently. HCM can present in early childhood with significant hypertrophy and LV outflow tract (LVOT) obstruction while in others may be asymptomatic into late adulthood (Ackerman 2005). These observations suggest that the disease course is not dependent solely on

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the pathogenic gene but is modified by other factors that can be environmental or genetic.

The renin–angiotensin–aldosterone-system (RAAS) contributes to ventricular hypertrophy through effects mediated by circulating angiotensin as well as local activation of RAAS in the myocardium (Kim and Iwao 2000). Angiotensin (Ang) I is produced from angiotensinogen (AGT) which is converted to Ang II predominantly by angiotensin-converting enzyme (ACE) and partly by chymase (CMA). Ang II binds primarily to Ang II type 1 receptor (AGTR1) to promote cell growth and hypertrophy. It is also converted to aldosterone by aldosterone synthase (CYP11B2) which promotes fluid retention and cardiac fibrosis (Pagliaro and Penna 2005). The presence of common genetic variants, or polymorphisms, in genes encoding the RAAS can enhance RAAS activation and/or receptor function. In studies in adults, polymorphisms in RAAS genes have been reported to enhance the LV hypertrophic response to exercise training, hypertension, left ventricular outflow tract (LVOT) obstruction, as well as HCM (Kurland et al. 2002; Diet et al. 2001; Ortlepp et al. 2002). In particular, polymorphisms in multiple RAAS genes can act synergistically on several steps in the RAAS pathway resulting in a compound effect on severity of LVH. This has been reported by Ortlepp et al. (2002) who demonstrated a correlation between number of RAAS gene polymorphisms and severity of LVH in a family of HCM. However, other studies have failed to demonstrate a consistent association between the RAAS genotype and severity of LVH, potentially due to a cross-sectional study design and a failure to evaluate the longitudinal effect of the RAAS genotype on disease progression.

Hypertrophic Cardiomyopathy presenting in childhood represents a particularly severe form of disease characterized by early onset and rapid progression (Maron 2004). In addition, since the growing heart shows a greater dependence on the RAAS for growth compared to the adult heart, it may be more susceptible to variations in the RAAS genotype (Sen and Rajasekaran 1991). The influence of RAAS genotype on progression of HCM in a growing population has not been previously studied. The objective of this study was to determine the association of polymorphisms in a composite of five RAAS genes with the severity and progression of cardiac hypertrophy in children with HCM. The following polymorphisms were evaluated: (1) a M235T missense mutation in the gene encoding angiotensinogen (*AGT*), (2) a deletion variant of the *ACE* gene, 287 base pair intron 16 (*DD*), (3) an A/G polymorphism at position −1,903 of the cardiac chymase A gene (*CMA*), (4) an A/C polymorphism at position 1,666 of the Ang II type 1 receptor gene (*AGTR1*), and (5) a C/T polymorphism at position −344 in the aldosterone synthase gene (*CYP11B2*) (Perkins et al. 2005).

Methods

Study population

The study was a prospective evaluation of the influence of RAAS genotypes on the progression of LVH in children with HCM. All children below age 21 years with HCM ($n = 65$) evaluated at Morgan Stanley Children's Hospital of New York Presbyterian or University of California San Francisco between 2001 and 2005 were eligible for the study. HCM was diagnosed by clinical criteria of LV wall thickness greater than normal for body surface area (BSA) in the absence of systemic causes of hypertrophy, or by the presence of a known sarcomere mutation identified on familial screening. The study was approved by the local Institutional Review Boards. Informed consent was obtained from the parents and assent was obtained in addition from older subjects.

Genotyping

Candidate gene polymorphisms in the RAAS neuro-hormonal axis were selected based on previous association studies, known functional effects of the polymorphisms and prevalence in published medical literature (Kurland et al. 2002; Diet et al. 2001; Ortlepp et al. 2002) RAAS genotype was determined in all 65 subjects. A single 5 ml venous blood sample was collected for isolation of genomic DNA from leukocytes by cell lysis followed by DNA extraction and precipitation according to manufacturer's instructions (Promega). Polymerase chain reactions (PCR) for amplification of DNA fragments consisted of 20 µl reaction volumes with 100 ng genomic DNA, 1× reaction buffer (Boehringer Mannheim) containing ($MgCl_2$) 1.5 mM, 0.25 mM each dNTP, 100 ng of each PCR primer (Table 1), and 1 U Taq polymerase. All thermocycling was performed with 35 cycles of denaturation at 94°C for 30 s, and annealing at 55°C, unless otherwise specified, for 30 s, and extension at 72°C for 30 s.

Pyrosequencing assays were performed for *AGTR1, CYP11B2, AGT*, and *CMA*. Pyrosequencing is a simple, quantitative method of sequencing short lengths of DNA by synthesis (Ronaghi and Uhlen 1998). Amplicons of approximately 200 bp were amplified from genomic DNA using a biotin labeled primer (Table 1) and subsequently purified using streptavidin beads. Short sequence reads off a sequencing primer internal to the original amplicon primer of approximately 8 bp produce real time signal by emission of light by luciferase fueled by ATP synthesized for the pyrophosphate released during dNTP incorporation. Genotyping was performed according to the manufacturer's recommended protocol (Biotage, Uppsala, Sweden).

Table 1 Primer sequences for the renin–angiotensin–aldosterone system genotype assays

Angiotensinogen	
AGT-F	ggtggtcaccaggtatgtcc
AGT-R	aggctgtgacaggatggaag
AGT-seq-F	tgctgtccacactggctccc
Angiotensin converting enzyme	
ACE3as	gccctgcaggtgtctgcagcatgt
ACE3as	ggatggctctccccgccttgtctc
ACE5a	tgggaccacagcgcccgccactac
ACE5c	tcgccagccctcccatgcccataa
Chymase	
CMA-F	ttccatttcctcaccctcag
CMA-R	cagaagagaatccggagctg
CMA-seq-F	caccctcagccaggcaggtg
Aldosterone synthase	
CYP11B2-F	tggagggtgtacctgtgtca
CYP11B2-R	tccagggctgagaggagtaa
CYP11B2-seq-R	cttatcgtgagatgagaggg
Angiotensin II receptor, type I	
AGTR1-F	agaagcctgcaccatgtttt
AGTR1-R	tgtggctttgctttgtcttg
AGT1-seq-F	cacttcactaccaaatgagc

For the angiotensin converting enzyme (*ACE*) assay, PCR products were electrophoresed through a 2% agarose gel stained with ethidium bromide. Alleles were read as insertions or deletions by their respective sizes of 597 and 319 bp. Because the deletion allele is preferentially amplified, each apparent deletion homozygote was subject to a second PCR assay using the primers ace5a and ace5c that recognized sequences specific to the insertion sequence to ensure that apparent deletion homozygotes were not actually insertion/deletion heterozygotes. A RAAS polymorphism known to be associated with a pro-hypertrophic response was defined as a pro-LVH polymorphism. Homozygosity for the pro-LVH allele constituted a pro-LVH genotype. Patients were further divided into two groups based on the number of pro-LVH homozygous RAAS genotypes per individual i.e., <2 and ≥ 2. The pro-LVH alleles are shown in Table 2.

Clinical and echocardiographic data

Data on age at presentation, gender, race, diagnosis, medications, family history, and surgical history were collected. Two-dimensional and M-mode echocardiography were performed using conventional methods at enrollment and yearly thereafter (Elliott et al. 2000). Magnitude of LVH was assessed primarily in the parasternal long and short-axis planes (Spirito et al. 2000). Chamber dimensions were measured according to published recommendations. (Sahn et al. 1978) LV end diastolic dimension (LVEDD), interventricular septal thickness (IVST) and posterior wall thickness (PWT) were measured at end diastole according to the recommendations of the American Society of Echocardiography and indexed to BSA. The greatest thickness measured at any site in the LV wall was considered to represent the maximal wall thickness. Asymmetric septal hypertrophy was defined as IVST greater than LV posterior wall thickness. LV mass was calculated using the area–length method. The volume of LV myocardium was estimated as the difference of volumes calculated from the outer (epicardial or RV septal border) and the inner outlines of the LV; the volume of the LV myocardium multiplied by muscle density (1.055 g/cm^3) yields the LV mass. LV mass was indexed to BSA (LVMI) and was also measured as LV mass *z*-score corrected for BSA. LV outflow tract velocities at rest were determined using continuous wave Doppler echocardiography, and LV outflow tract gradients were calculated using the modified Bernoulli equation. Patients were followed longitudinally and the change in echocardiographic measures of LVH was determined from baseline for each patient. In enrolled patients that died, were transplanted, or underwent myectomy during the study period, data from the last echocardiogram prior to death/transplant/surgical intervention were included in longitudinal analysis. The echocardiograms were reviewed by a primary echocardiographer blinded to the subjects' clinical status and RAAS genotype.

Table 2 Renin–angiotensin–aldosterone system pro-LVH allele frequency ($n = 65$)

Gene	"Pro-LVH" allele frequency[a]
Angiotensinogen	$G = 0.60$
Angiotensin converting enzyme	$D = 0.54$
Chymase	$A = 0.39$
Angiotensin II type 1 receptor (AT1R1166)	$C = 0.20$
Aldosterone synthase	$G = 0.37$

LVH left ventricular hypertrophy

[a] Comparable to frequencies in general population and in previously published studies (Ortlepp et al. 2002; Perkins et al. 2005) ($P > 0.05$ by Chi-square analysis for all genes)

Statistical analysis

Sample size calculations

The anticipated percent change in LVM *z*-score during one year follow-up for the entire cohort was estimated at $30 \pm 45\%$ based on our preliminary data. A total of 40

subjects (20 per arm) were required to detect an absolute difference of 40% in change in LVM z-score between patients with ≥2 homozygous pro-LVH genotypes and <2 homozygous pro-LVH genotypes with 80% power using a two-sided 0.05 level test. This sub-grouping was based on the finding of a median frequency of 1 pro-LVH RAAS genotype in our population. A total of 65 subjects with HCM were enrolled into the study to ensure a target sample size of 40 subjects based on the anticipated frequency of polymorphisms and anticipated loss to follow-up.

To determine whether the genotypes were in Hardy–Weinberg equilibrium, actual and predicted genotype counts were compared by Chi-square analysis. The overall goal was to assess if RAAS genotype was associated with clinical phenotype in children with HCM at presentation and during follow-up. The primary outcome measure was change in LVM z-score and IVST from baseline to last follow-up. Clinical and echocardiographic variables at first evaluation were compared between subjects with ≥2 versus <2 pro-LVH RAAS genotypes using the non-parametric Mann–Whitney test, Chi-square test, and Fisher exact test. Mann–Whitney test and paired Student's T test were used for between and within group comparisons of change in LVM and IVST during follow-up. Univariate linear regression models, based on maximum likelihood estimates, were created to assess the independent effect of the RAAS genotypes, age, gender, race and positive family history on outcome measures. A linear regression model, also based on maximum likelihood estimates, was created in which each individual RAAS gene and positive family history were included in order to test for independent effect. High-risk genotypes and/or familial antecedent with P values in the multivariate model under 0.05 were deemed significant associations. These models were also used to test a possible effect of heterozygosity. All statistical analyses were performed using SPSS version 14.0 for Windows and SAS v.9.1 (The SAS Institute, Cary, NC, USA).

Statement of responsibility

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Population

The study population consisted of 65 children with HCM, of which 62 were unrelated and only 3 were related family members. Mean age at presentation was 9.6 ± 6 years (range 0–20 years); 62% were male. Family history was positive for HCM in 29 (44%) patients. The etiology of HCM was identified in 14 patients and included 12 patients with known sarcomeric mutations: β myosin heavy chain ($n = 3$), myosin binding protein C ($n = 1$), myosin heavy chain 7 ($n = 6$), troponin 2 ($n = 2$). Two patients had Noonan syndrome. Racial/ethnic distribution of the subjects was: Caucasian 43%, Black 23%, Hispanic 20%, Asian 14%.

Frequency of polymorphisms

The frequency of the RAAS polymorphisms in the HCM cohort was similar to that previously reported in other study populations and was in Hardy–Weinberg equilibrium for each gene ($P > 0.05$ by Chi-square analysis for all genes) (Table 2) (Ortlepp et al. 2002; Perkins et al. 2005). Forty percent patients were homozygous for the *AGT* M235T, 34% were homozygous for the *ACE* D/D polymorphism, 17% were homozygous for the *CMA* AA genotype, and 9% each for the *AGTR1* CC and *CYP11B2* CC genotypes. Median number of homozygous pro-LVH RAAS genotypes in the study population was 1. Nineteen (29%) patients had ≥2 pro-LVH and 46 (71%) patients had <2 pro-LVH RAAS genotypes.

Genotype–phenotype correlation

Clinical characteristics

The clinical characteristics of patients divided by number of pro-LVH RAAS genotypes are shown in Table 3. There were no significant differences between the genotype groups in age, gender, race, etiology of HCM or medication use at initial evaluation. Five patients were receiving β-blocker and/or calcium-channel blocker at initial evaluation. The mean duration of follow-up was 2.8 ± 3 years (median 1.5 years). Seven patients were receiving β-blocker and/or calcium-channel blocker therapy at last follow-up. Ten patients underwent myectomy for significant LV outflow tract obstruction during follow-up. One patient died and one patient received a heart transplant. Subject age, gender, length of follow-up, medication use, and need for surgical interventions were not different between the <2 and ≥2 genotype groups.

Echocardiographic characteristics

Echocardiographic measures of LV dimensions and function for the entire cohort at presentation were as follows: septal thickness 1.4 ± 0.1 cm/m^2, indexed LV mass 140 ± 12 g/m^2, LV mass z-score 3.4 ± 0.4, LV shortening fraction 44 ± 11%, and mean LVOT gradient 25 ± 40 mmHg. Nineteen (29%) patients had LVOT gradient

Table 3 Clinical characteristics across RAAS genotypes ($n = 65$)

	No pro-LVH genotypes	1 pro-LVH genotype	2 pro-LVH genotypes	3 pro-LVH genotypes
Number	18 (28%)	28 (43%)	14 (21%)	5 (8%)
Gender: male (%)	72	50	79	40
Age at presentation (years)	7.8 ± 5	11.0 ± 6	8.1 ± 6	11.9 ± 6
Body surface area (m^2)	0.9 ± 0.4	1.3 ± 0.6	1.1 ± 0.7	1.4 ± 0.6
Race (%)				
Caucasian	55	36	36	60
Black	11	25	29	40
Hispanic	17	25	21	0
Asian	17	14	14	0
Positive family history (%)	47	46	36	40
Myectomy	1	5	4	0
Transplant	0	2	1	0
Died	0	1	0	0

LVH LV hypertrophy

Table 4 Echocardiographic characteristics based on RAAS genotype ($n = 40$)

	Baseline		Follow-up	
	<2 pro-LVH genotypes	≥2 pro-LVH genotype	<2 pro-LVH genotypes	≥2 pro-LVH genotype
Number	46 (71%)	19 (29%)	29 (73%)	11 (27%)
IVST (cm/m^2)	1.4 ± 0.2	1.5 ± 0.3	1.1 ± 0.1#	1.7 ± 0.4#
LV mass indexed (g/m^2)	147 ± 18	186 ± 39*	164 ± 16	251 ± 50#
LV mass z-score	2.5 ± 0.6	5.2 ± 1.3*	4.4 ± 0.6#	7.4 ± 1.2#
LV SF (%)	43 ± 2	45 ± 3	41 ± 2	49 ± 3*
Asymmetric septal hypertrophy	41%	58%*	28%#	73%*#
LV outflow peak gradient (mmHg)	24 ± 7	29 ± 9	14 ± 8	51 ± 17*
LV outflow gradient >30 mmHg	25%	42%*	7%	45%*

LVH LV hypertrophy; *SF* shortening fraction; *IVST* inter-ventricular septal thickness

*$P < 0.05$ compared to subjects with <2 pro-LVH RAAS genotypes

#$P < 0.05$ compared to baseline

greater than 30 mmHg. Complete serial echocardiographic data were available in 40 subjects during study follow-up including 29 patients with <2 pro-LVH RAAS genotypes and 11 patients with ≥2 pro-LVH RAAS genotypes. Data post myectomy were not included in the analysis. Results for both independent group comparisons of echocardiographic data at last follow-up, as well as paired data for subjects within each group are reported. The baseline and follow-up echocardiographic characteristics based on number of pro-LVH genotypes are shown in Table 4.

(i) Indexed LV mass and LV mass *z*-score were significantly higher in subjects with ≥2 pro-LVH genotypes compared to those with <2 both at initial evaluation and follow-up with the former showing a 35% increase in LVMI during follow-up while LVMI did not change significantly in the <2 genotype group (Fig. 1a). (ii) IVST was not different between the two groups at initial evaluation but increased significantly only in the ≥2 pro-LVH genotype patients on follow-up ($P < 0.05$) (Fig. 1b). The IVST increased by 22 ± 16% in those with ≥2 polymorphisms but decreased by 16 ± 5% in those with <2 polymorphisms. This difference in IVST change between the two groups was significant ($P = 0.04$) (Fig. 2a). More subjects in the ≥2 pro-LVH genotype group had asymmetric septal hypertrophy compared to those in the <2 group both at initial evaluation and follow-up ($P < 0.05$) (Fig. 2b). (iii) Increased septal hypertrophy was associated with a higher frequency of LV outflow tract obstruction in patients with ≥2 pro-LVH RAAS genotypes i.e., LV outflow gradient >30 mmHg at rest compared to those with <2 pro-LVH genotypes ($P < 0.0001$) and higher LVOT gradient ($P < 0.05$). (iv) The rapid increase in LVM and IVST was also seen when patients were divided into risk groups based on number of pro-LVH RAAS alleles. Subjects with ≥5 pro-LVH RAAS alleles showed a greater increase in LVMI (37 ± 15%, $P = 0.02$) compared to those with <5 alleles (9 ± 8%, P = NS). Changes in IVST were also greater in the ≥5 allele compared to the <5 allele group. Similar results were seen with LVM *z*-score.

On multivariate analysis, a pro-LVH RAAS genotype was associated with a higher LV outflow tract gradient

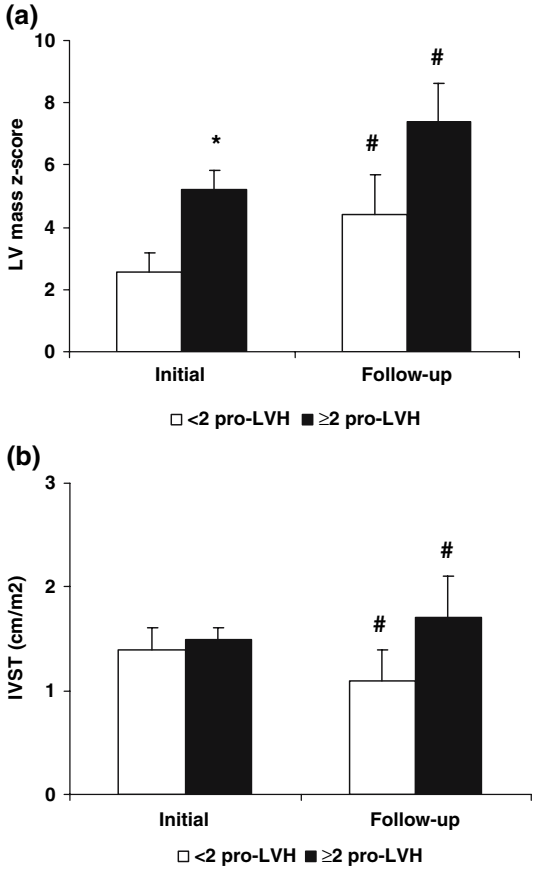

Fig. 1 **a** LV mass *z*-score higher in patients with ≥2 ($n = 11$) (*black bars*) compared to those with <2 pro-LVH RAAS genotypes ($n = 29$) (*white bars*), both at baseline and during follow-up. LV mass increased significantly in both groups during study follow-up. **b** Septal thickness (IVST) was not different between the two genotype groups at baseline. On follow-up, IVST decreased in patients with <2 pro-LVH RAAS genotypes compared to a significant increase in patients with ≥2 pro-LVH genotypes. *$P < 0.05$ between the two groups; #$P < 0.05$ from baseline to follow-up. *IVST* inter-ventricular septal thickness; *LVH* LV hypertrophy; *RAAS* Renin–angiotensin–aldosterone system

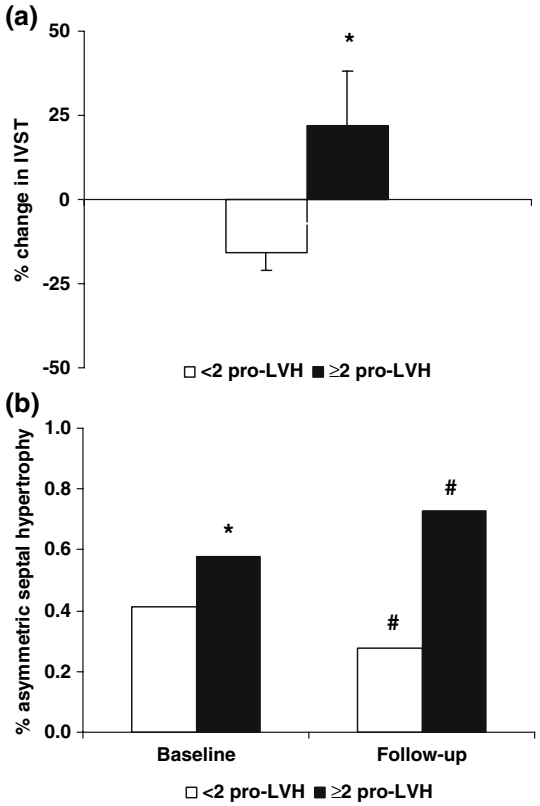

Fig. 2 **a** Septal thickness (IVST) decreased on follow-up in patients with <2 pro-LVH RAAS genotypes (*white bars*) but increased in those with ≥2 genotypes (*black bars*) ($P < 0.05$). **b** This was associated with a greater incidence of asymmetric septal hypertrophy (septal thickness greater than LV free wall thickness) in patients with ≥2 pro-LVH RAAS genotypes ($n = 19$) (*black bars*) compared to those with <2 pro-LVH genotypes ($n = 46$). This worsened during follow-up in the ≥2 genotype group ($n = 11$) but decreased in the <2 group ($n = 29$). *$P < 0.05$ between the two groups; #$P < 0.05$ from baseline to follow-up. *IVST* inter-ventricular septal thickness; *LVH* LV hypertrophy; *RAAS* Renin–angiotensin–aldosterone system

[Odds ratio, 1.47 (95% confidence intervals, 1.16–1.86), $P = 0.002$], greater septal thickness [Odds ratio, 1.90 (1.13–3.22), $P < 0.05$], and larger increase in LV mass [Odds ratio, 1.40 (1.02–1.93), $P < 0.05$] and IVST [Odds ratio, 1.46 (1.15–1.85), $P < 0.05$] on follow-up. A higher number of pro-LVH genotypes was associated with a larger effect size ($P < 0.05$). Of all the RAAS genotypes, the pro-LVH CYP genotype demonstrated the strongest association with progression of LVH ($P < 0.05$). There was a positive correlation between the number of pro-LVH RAAS genotypes and increase in LV mass ($r = 0.27$, $P = 0.05$) and with increase in IVST during follow-up ($r = 0.41$, $P = 0.009$). The number of pro-LVH alleles also correlated positively with percent change in LV mass over time ($r = 0.33$, $P = 0.038$).

Race, gender, and age at initial presentation did not significantly influence the observed effect of the RAAS genotype on LVH. A positive family history was associated with a smaller increase in LV mass and IVST at follow-up ($P < 0.05$). However, there was no interaction between the RAAS genotype and family history i.e., the RAAS genotype and family history were independent predictors of disease progression.

Discussion

This study investigated the association between functional variants in five genes of the RAAS neuro-hormonal axis previously implicated in ventricular hypertrophy, with the severity and progression of LVH in children with HCM. The presence of more than one pro-LVH RAAS genotype was associated with increased LVOT obstruction, septal hypertrophy and progression of HCM.

This effect was independent of the underlying cause of HCM. This is the first study to evaluate the effect of RAAS genotype on progression of HCM in children and to identify a potential genetic determinant of the obstructive phenotype. There are several important findings of this study.

Effect on LVH

A pro-LVH RAAS genotype was associated with a higher LV mass *z*-score at initial evaluation and at last follow-up. This appeared to be secondary at least in part to a disproportionate increase in septal thickness and in resting LVOT gradient. Several studies have identified the presence of outflow tract obstruction as a risk factor for poor outcomes in HCM (Maron et al. 2003; Ommen et al. 2005). Maron et al. (2003) reported a twofold higher risk of death and a fourfold higher risk of progression to symptomatic heart failure in HCM patients with outflow obstruction compared to those without. Twenty-five percent of patients in their study had a peak instantaneous gradient of >30 mmHg under basal (resting) conditions which is comparable to the 29% frequency in our patient cohort. These studies did not identify predictors of progressive LV outflow tract obstruction. Our study is the first study to provide a possible genetic basis for a predisposition to an obstructive phenotype, likely related to the progressive septal hypertrophy in patients with the high risk RAAS genotype.

Compound effect of RAAS polymorphisms

Another important finding was that the compound effect of the RAAS gene polymorphisms was larger than that of any individual RAAS gene i.e., having more than one pro-LVH RAAS polymorphism had a greater impact on disease progression than any individual RAAS gene. Most studies have investigated the influence of polymorphisms in a single RAAS gene with often conflicting results (Lechin et al. 1995; Lopez-Haldon et al. 1999; Osterop et al. 1998; Pfeufer et al. 1996). Our findings emphasize the importance of analyzing multiple genes in a biologic pathway that can act additively or synergistically at different levels in the pathway. The CYP11B2 polymorphism i.e., aldosterone synthase gene polymorphism was associated with the strongest effect. Upregulation of this enzyme is associated with increased aldosterone production which promotes myocardial fibrosis (Chai and Danser 2006; Heymes et al. 2004). Progressive myocardial fibrosis would result in progressive ventricular remodeling and hypertrophy, which could provide a potential mechanism for the progressive septal hypertrophy and increase in ventricular mass seen in these patients.

Interaction between RAAS genotype and other risk factors

A third important finding of our study is that the association of the RAAS genotype with disease progression can be generalized to a population of unrelated patients with HCM with different sarcomeric gene mutations as well as to those with non-sarcomeric forms of HCM and not just to families with the myosin binding protein C (MyBP-C) mutation. Two previous studies reported an association of the RAAS genotype with the severity of HCM only in individuals with HCM caused by a mutation in the MyBP-C (Ortlepp et al. 2002). In particular, Perkins et al. (2005) in a cross-sectional study of 389 unrelated adult patients, reported a pro-hypertrophic effect of the *ACE* DD genotype only in the subset of patients with a mutation in MyBP-C. Our results showed that the RAAS genotype was an independent predictor of disease progression, independent of the underlying disease etiology. The ability to see an effect of the RAAS genotype across etiologies may be related to the following differences in our study: (i) a longitudinal study design that did not rely on a single time point to assess for differences in this phenotypically heterogeneous disease, (ii) the younger age of the patients who may be more susceptible to the effects of variations in RAAS activation, and (iii) analysis of several RAAS genes rather than a single RAAS gene.

A positive family history was an independent predictor of disease progression but with opposing effects i.e., a positive family history was associated with slower progression. This may reflect the fact that familial HCM is often diagnosed early by screening of asymptomatic family members whereas sporadic or non-familial HCM is usually only diagnosed when a patient becomes symptomatic from progressive disease. Also, non-familial HCM may reflect disease caused by factors other than known sarcomeric mutations, which may independently affect rate of disease progression. Notwithstanding, family history did not influence the effect of the RAAS genotype on LVH i.e., the RAAS genotype was associated with disease severity regardless of the etiology of HCM. The finding that the RAAS genotype can predict disease progression in patients with HCM regardless of age, gender and etiology has important implications for risk stratification in this population.

Influence of RAAS genotype in a growing population

Finally, an important finding of our study is the influence of RAAS polymorphisms in childhood HCM and young adults (<21 years old) which has not been previously studied. Understanding disease mechanisms in a growing population is critical since early onset HCM appears to represent a more severe phenotype of HCM. Young age has been reported as an independent predictor of poor outcome in

patients with HCM (Sorajja et al. 2006). This is particularly true of the infant age group (Colan et al. 2007). The adolescent period is also a particularly vulnerable period since HCM often manifests during the accelerated growth spurt of adolescence (Maron 2004). Given that RAAS is an important mediator of cardiac growth, the growing heart may be more susceptible to variations in the RAAS genotype. In our study, pediatric patients with HCM showed rapid progression of LVH but this was most marked in patients with $\geq$2 pro-LVH RAAS genotypes. Those with <2 pro-LVH RAAS genotypes showed no significant change in LV mass during the same time period but showed an actual decrease in septal hypertrophy, reflecting reverse remodeling in these subjects. This suggests that having a low risk RAAS genotype may actually have a protective effect on disease progression in this relatively high-risk population of growing children.

Limitations

(i) While our study did not assess the effect on clinical outcomes, several studies have consistently shown that LV mass and septal hypertrophy are independent predictors of mortality not only in patients with cardiomyopathy but also in patients with other cardiovascular risk factors (Schillaci et al. 2000). We therefore used increase in LV mass and IVST as surrogate outcomes in our study. (ii) There may be intra-familial differences in disease progression depending on the genetic etiology of HCM. However, the family sizes were too small to determine the influence of RAAS genotype within individual families with HCM. Also while 44% of subjects had familial HCM, there were only 3 family members with HCM represented in this cohort. It is therefore unlikely that inclusion of family members significantly confounded our results. (iii) We only measured resting LV outflow tract gradient since exercise testing was not routinely performed in this population. This may have underestimated the number of patients with obstruction that manifests only during exercise. (iv) It was not possible to measure tissue RAAS activity in the different genotype groups since patients do not undergo routine myocardial biopsies.

Clinical relevance

Hypertrophic cardiomyopathy is the most common cause of sudden death in the young (Maron et al. 1995). Our study identified a pro-LVH RAAS genotype as a significant risk factor for rapid disease progression in children with HCM. In particular, the RAAS genotype was associated with progressive septal hypertrophy and increased severity of LV outflow obstruction, both of which are risk factors for poor outcomes including risk for sudden death. This suggests that patients with pro-LVH RAAS polymorphisms may need closer monitoring for development of LV obstruction and earlier evaluation for septal myectomy in selected cases. Studies in adults with heart failure or hypertension have shown that patients with pro-LVH RAAS polymorphisms show greater reduction in LV mass in response to ACE inhibitors or angiotensin receptor blockers (McNamara et al. 2004; Kurland et al. 2002). Our results suggest a need for clinical trials to evaluate the efficacy of ACE inhibitors, angiotensin-receptor blockers or aldosterone receptor blockers in HCM patients with a high risk RAAS genotype.

In conclusion, early identification of genetic risk factors can have important clinical implications for risk stratification as well as the development of strategies for closer surveillance and intervention tailored to the individual's risk profile.

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