Association between tyrosine hydroxylase polymorphisms and left ventricular structure in young normotensive men

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ABSTRACT

Tyrosine hydroxylase (TH) is a rate-limiting enzyme for catecholamine biosynthesis. Increased sympathetic activity is associated with an increased left ventricular (LV) mass. However, the influence of TH gene polymorphisms on LV structure and function has yet to be investigated. Here, we analyse the association of Val-81-Met and tetranucleotide TCAT repeat TH polymorphisms with LV structure and function (assessed by echocardiography) in 108 normotensive men aged ≤ 35 years (mean age: 25±4 years) with body mass index (BMI) ≤ 30 kg/m^2 (mean BMI: 23±3 kg/m^2). The distribution of genotypes was VV homozygotes (n=42), VM heterozygotes (n=49) and MM homozygotes (n=17). The Val-81-Met polymorphism showed significant linkage disequilibrium with the TCAT polymorphism (P<0.0001). No differences were seen between the subgroups with respect to age, BMI and blood pressure. Compared with the VV and VM genotypes, subjects with the MM genotype showed significantly (all P<0.05) increased LV cavity diameter (VV: 52.8±3.9 mm, VM: 52.9±3.6 mm, MM: 56.1±3.2 mm), global LV mass (VV: 159±31 g, VM: 165±36 g, MM: 187±30 g) and LV mass index (VV: 81±14 g/m^2, VM: 84±17 g/m^2, MM: 93±12 g/m^2). No differences were seen between the subgroups in parameters of LV function. In addition, plasma epinephrine and norepinephrine levels were comparable in the three subgroups. The results suggest an important association between the MM genotype of Val-81-Met TH gene polymorphism and increased LV cavity dimension and mass in a young normotensive male population, indicating an important role for genetic determination of the sympathetic system in LV growth.

KEY WORDS: Catecholamines. Polymorphism (genetics). Tyrosine 3-monooxygenase. Ventricular structure, left.

Introduction

Left ventricular (LV) mass is an independent predictor of an increased prevalence of life-threatening arrhythmias, sudden death, coronary artery disease, heart failure and stroke.[1,2] In addition to well-recognised haemodynamic stimuli for LV hypertrophy, such as pressure and volume overload, it is recognised that non-haemodynamic stimuli such as neurohormonal factors and genetic predisposition are involved in the genesis of LV hypertrophy. Genetic factors may play an important role in the determination of myocyte response to increased workload and in the regulation of the neurohormonal systems and tissue responses to humoral effectors.

Many studies have investigated a potential association between multiple candidate gene polymorphisms and LV hypertrophy. Mostly, these genes encode components of the renin-angiotensin system.[3-6] However, the sympathetic nervous system is also known to play an important role in the genesis of LV hypertrophy. Norepinephrine causes myocardial hypertrophy in cultured cardiomyocytes[7] and in animal models,[8] and thus is considered a 'myocardial hypertrophy hormone'.[9]

In humans, cardiac norepinephrine release is strongly associated with increased LV mass, both in hypertensive patients and in normotensive subjects.[10] Therefore, the gene encoding tyrosine hydroxylase (TH), the initial and rate-limiting enzyme for catecholamine biosynthesis, may be considered a candidate gene for many cardiovascular disorders including LV hypertrophy.[11,12]

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In this study we focus on a TH gene polymorphism that results in a valine for methionine amino acid change at codon 81 (Val-81-Met) within exon 2 of the gene. Furthermore, we test the association of this polymorphism with TCAT repeat microsatellite polymorphism of the same gene, which influences the gene transcription and is linked with essential hypertension.[12]

To investigate whether or not Val-81-Met TH polymorphism influences cardiac structure and function, echocardiography and DNA analysis is performed on a population of young, healthy normotensive men. This

particularly homogenous population, recruited from families participating in a large population survey, was chosen to avoid any misleading influence of gender, age or associated pathological conditions.[13]

Materials and methods

Study population

Two hundred and nine normotensive men (blood pressure <140/90 mmHg) were recruited from a large transversal study conducted in the department. All were without clinically apparent metabolic and cardiovascular disease. Competition and high-level athletes were excluded from the study. From this group of 209, 145 had previously undergone a complete echocardiographic study. In the present study, we included men aged between 18 and 35 years (n=124) who gave their consent for DNA analysis (n=118).[13] DNA analysis for TH polymorphism was available in 116 subjects. Five overweight subjects (body mass index [BMI] >30 kg/m^2) were excluded, along with three subjects with asymmetric septal hypertrophy. Therefore, the final analysis population consisted of 108 subjects.

Echocardiography

Echocardiography had been performed three years earlier than TH polymorphism assessment and thus the operator would not have known the results of subsequent DNA analysis. All examinations were performed using a Toshiba 160 SSH-A echocardiography machine with 2.5 MHz phased array probe. A single operator performed most of the examinations. In all subjects, thickness and diameters were measured according to the recommendations of the American Society of Echocardiography,[14] using the M-mode recording. Measurements for interventricular septum (IVSd), posterior wall (PWd) and internal LV diameter (LVDd) were taken, and left atrial diameter (LA) was measured at the moment the aortic valve closed. All were performed online at least three times and averaged.

Asymmetric septal hypertrophy was defined as an IVSd >13 mm and IVSd:PWd ratio >1.5. LV mass was calculated according to the Devereux-modified ACE cube formula and indexed to body surface area (BSA).[15] Stroke volume (SV), cardiac output (CO) and ejection fraction (EF) were calculated using Teichholz's formula.[14] Doppler parameters of transmitral filling were recorded using the pulsed-wave mode, with sample volume located at the level of the tips of the mitral valve leaflets. Peak E and A velocities were measured and the E:A ratio calculated.[16]

Polymorphism assessment and catecholamine measurement

Genomic DNA was isolated from peripheral blood leucocytes and two polymorphisms of the TH gene were detected using a polymerase chain reaction (PCR) technique.[11] The TCAT repeat polymorphism was determined with primers 5'-GGCAAATAGGGGGCAAAA-3' and 5'-GGCTTCCGAGTGCAGGTC-3'.

Each 25-µL reaction contained 200 ng genomic DNA, 1.2 µmol/L each primer, 0.2 mmol/L each dNTP and 0.5 units *Thermus aquaticus* (*Taq*) DNA polymerase (Roche Diagnostics, D-68298 Mannheim, Germany). Reactions were incubated at 95°C for 4 min, followed by 30 cycles at 56°C for 30 sec, 72°C for 30 sec, 92°C for 30 sec, and one cycle each at 56°C for 30 sec and at 72°C for 10 min. Resulting fragments were separated using electrophoresis on a 4.7% Metaphor agarose gel stained with ethidium bromide.

All samples were reamplified and genotype was confirmed without knowing the previous result. All alleles were assigned by a single observer. We have identified five alleles for this polymorphism: 144 bp (allele A), 148 bp (B), 152 bp (C), 156 bp (D) and 160 bp (E).[11]

The primers used for the Val-81-Met polymorphism were 5´-GGAGAAGGAGGGGATGGCC-3´ and 5´-ACCAGCTCACCTCAAACACCT-3´.[15] Each 25-µL reaction contained 300 ng genomic DNA, 1.0 µmol/L each primer, 0.2 mmol/L each dNTP, and 0.6 units *Taq* DNA polymerase. The reactions were incubated at 95°C for 4 min, followed by 33 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and one cycle at 72°C for 5 min. The PCR products (101 bp) were incubated subsequently with BalI restriction enzyme, which recognises the Met, but not the Val, sequence. The resultant products were the V and M alleles

For plasma catecholamine estimation, blood was collected from an indwelling cannula after 15 min of supine rest and immediately before the echocardiographic examination. Epinephrine and norepinephrine levels were measured using a commercial radioenzymatic kit (Immunotech, Prague, Czech Republic).[11]

Statistical analysis

Statistical analysis was performed using JMP statistical software (SAS Institute Inc.). Group data were expressed as mean ± SD. Differences between multiple groups were tested by using analysis of variance (ANOVA) and differences between the groups were tested using the Kruskal-Wallis test. Linear regression analysis was used to assess the relationship between plasma catecholamines and LV structure. Furthermore, a multivariate regression analysis was used to assess the interaction between the studied polymorphism and other studied variables. P<0.05 was regarded as significant.

Results

Principal characteristics of the study population by TH Val-81-Met polymorphism are listed in Table 1. There were no significant differences between the subgroups due to age, BMI or BP level. Subjects with the MM genotype tended to have a slightly slower heart rate.

Table 2 shows echocardiographic parameters by TH Val-81-Met polymorphism. The differences in septal and parietal thickness were only small and not significant. Conversely, MM genotype was associated with a larger internal cavity diameter, global left ventricular mass, and LV mass index, and subjects with this genotype also had a significantly increased stroke volume. However, due to the trend towards slower heart rate in the MM subgroup (Table 1), the cardiac output did not differ significantly from MV and VV genotype subgroups. LV systolic function and diastolic filling were comparable in all three subgroups.

No significant differences were found between the subgroups in plasma epinephrine and norepinephrine levels (Table 3). Correlations between plasma catecholamines and LV parietal thickness and internal cavity diameter were not significant. Correlations between LV mass index and both

Table 1. Principal characteristics of the study population by tyrosine hydroxylase polymorphism

	Tyrosine hydroxylase polymorphism			*P* value
	VV (*n*=42)	VM (*n*=49)	MM (*n*=17)	(ANOVA)
Age (years)	25±4	25±4	24±5	NS
BMI (kg/m^2)	23±3	23±3	25±3	NS
BSA (m^2)	2.0±0.1	1.9±0.1	2.0±0.2	NS
Blood pressure (mmHg)				
Systolic	118±12	118±12	119±12	NS
Diastolic	79±9	79±8	78±6	NS
Heart rate (bpm)	72±15	68±8	65±9	0.07

Table 2. Left ventricular structural and functional parameters according to tyrosine hydroxylase polymorphism

	Tyrosine hydroxylase polymorphism			*P* value
	VV (*n*=42)	VM (*n*=49)	MM (*n*=17)	(ANOVA)
IVSd (mm)	9.0±1.3	9.1±1.5	9.1±1.4	NS
LVPWTd (mm)	7.8±1.1	8.0±1.0	8.1±1.0	0.10
LVDd (mm)	52.8±3.9	52.9±3.6	56.1±3.2**	0.005
LVM (g)	159±31	165±36	187±30**	0.02
LVMi (g/m^2)	81±13	85±17	93±12*	0.02
EF (%)	68±6	68±8	67±7	NS
SV (mL)	93±17	95±19	107±22**	0.03
CO (L/min)	6.7±1.6	6.5±1.6 7.	0±2.0	NS
E (m/s)	0.83±0.16	0.86±0.16	0.90±0.15	NS
A (m/s)	0.48±0.12	0.46±0.14	0.47±0.11	NS
E:A	1.8±0.6	2.0±0.6	2.0±0.7	NS
LA (mm)	35±4	35±5	36±4	NS

IVSd = interventricular septum thickness in diastole, PWd = left ventricular posterior wall thickness in diastole, LVDd= left ventricular internal diameter in diastole, LVM = left ventricular mass, LVMi = left ventricular mass index, EF = ejection fraction, SV = stroke volume, CO = cardiac output E = peak E wave velocity, A = peak A wave velocity, LA = left atrium. * $P<0.05$ *vs*. VV genotype, ** $P<0.05$ *vs*. VV and VM genotypes.

plasma epinephrine and norepinephrine were only slightly stronger, but they did not reach statistical significance (r=0.17, $P=0.13$ and r=0.20, $P=0.06$, respectively).

In multivariate analysis of Val-81-Met polymorphism, age, systolic BP and plasma norepinephrine level, only Val-81-Met genotype ($P<0.01$) and systolic BP ($P=0.05$) were significantly associated with LV internal cavity diameter ($r^2=0.13$, $P<0.05$). Furthermore, in the same model, only the Val-81-Met polymorphism was associated with LV mass index ($r^2=0.10$, $P=0.05$).

Val-81-Met and TH TCAT polymorphisms were in linkage disequilibrium, as conditional probabilities that the haplotype carried the Val allele when the TCAT allele was A, B, C, D or E were 0.97, 0.17, 0.81, 0.22 or 0.97, respectively ($\chi^2=67.5$, $P<0.0001$). The five alleles of the TCAT TH polymorphism separated our population into 15 subgroups, each containing only a small number of individuals. Therefore, the association of this polymorphism with LV structural parameters was not analysed.

Discussion

This study suggests that the Val-81-Met polymorphism had an important role in determining LV structure in a population with no other haemodynamic stimuli of cardiac growth. Results showed that the MM genotype of Val-81-Met TH polymorphism is associated mainly with an increase in LV chamber size. Despite the fact that the MM genotype was not accompanied by a significant increase in septal and parietal thickness, larger cavity size resulted in an increase in overall LV mass and LV mass index, compared to that seen with the VM and VV genotypes.

Subjects with the MM genotype also had an increased

Table 3. Plasma catecholamines concentrations according to tyrosine hydroxylase polymorphism

	Tyrosine hydroxylase polymorphism			*P* value
	VV (*n*=42)	VM (*n*=49)	MM (*n*=17)	(ANOVA)
Norepinephrine (nmol/L)	1.31±1.1	1.41±1.11	1.53±1.10	NS
Epinephrine (nmol/L)	0.32±0.26	0.31±0.19	0.30±0.13	NS

stroke volume but, due to a slightly slower heart rate, there were no significant differences in cardiac output. The increased stroke volume, most probably, was due to an increased LV cavity volume, as systolic ejection fraction was comparable in all three genotypes.

Association analysis demonstrated significant linkage disequilibrium with the TCAT repeat TH polymorphism; however, the number of individuals included in the study did not permit assessment of the association of this polymorphism with LV structure. However, we cannot exclude this polymorphism, previously shown to be associated with arterial hypertension,[12] as the primary cause of the TH functional modification leading to LV structural changes observed with the Val-81-Met polymorphism. This possibility should be investigated in the future, as the microsatellite polymorphisms of the TH gene are known to influence the gene transcription *in vitro*. [17]

Here, we studied a population of young, healthy normotensive men. The inclusion criteria were selected to avoid other possible influences on LV mass, such as gender, high BP, age and associated pathological conditions. Also excluded were overweight subjects, in whom echocardiogram is seldom of good quality and correction of LV mass for body size is difficult,[18] and individuals with asymmetric septal hypertrophy. In this latter category the geometrical hypothesis for LV mass calculation from M-mode measurements does not fully apply and the differentiation from monogenic familial hypertrophic cardiomyopathy might be difficult.

In addition, competition athletes were excluded; however, as TH polymorphisms are associated with several psychiatric disorders,[19] we cannot exclude the possiblilty that the polymorphisms are related to behavioral patterns that lead to more or less important daily physical activity that would influence LV structure. This, of course, remains purely hypothetical and merits further investigation.

Despite these limitations, and the small sample size, we believe our observation represents a genuine association, as the nature of the population studied makes an important selection bias unlikely.

The underlying mechanism responsible for the association of MM genotype with increased LV size and mass remains unclear. The subgroups defined by the genotype did not differ with regard to the usual determinants of LV mass, such as BP or body build. LV mass is associated with signs of enhanced sympathetic nervous system reactivity[20] and cardiac norepinephrine balance,[10] both in normotensive and hypertensive individuals. Human LV hypertrophy also is associated with disturbances in β-adrenergic receptor density[21] and impaired cardiac sympathetic innervation.[22] If TH polymorphism is associated with structural LV changes, it is possible that it is accompanied by an increase in sympathetic activity; an hypothesis supported by the findings of increased TH messenger RNA in spontaneously hypertensive rats,[23] and the association of TH gene microsatellite markers to essential hypertension[12] and its regulatory role in gene transcription.[17]

Simple increase in sympathetic activity does not fit with our observations. Other studies have shown that patients with sympathetic activation have higher heart rate and BP levels[24] and supernormal LV systolic performance on echocardiography.[25] In the present study, MM genotype was accompanied by a strictly normal BP, and LV ejection fraction and heart rate tended to be even slower than in other genotypes. Furthermore, chronic norepinephrine infusion in animals leads to a form of hypertrophy similar to that seen in volume overload[8] and leads to a decrease in heart rate,[26,27] while maintaining systolic and diastolic LV performance[26] – as observed in MM genotype subjects in our study. Animal studies show that norepinephrine is able to produce LV hypertrophy, even in the absence of any marked elevation of BP levels.[27] These data suggest that norepinephrine elevation has an important role in the genesis of LV hypertrophy.

In the present study, only negligible differences in catecholamine concentration between the genotype subgroups were observed, with just a weak and non-significant relationship between peripheral catecholamine concentration and LV structure. Unfortunately, venous catecholamine concentration does not necessarily reflect cardiac sympathetic nerve activity. Even when determining net cardiac catecholamine balance by arteriovenous concentration difference assessment, it is impossible to discern the relative impacts that the determinants of norepinephrine balance (sympathetic nerve density, firing rate, norepinephrine uptake, capillary permeability, organ mass, and blood flow) have.[10] Therefore, even in the absence of an association between plasma catecholamines and genotype and LV parameters, we cannot exclude the possibility that the link between MM genotype and LV structure is mediated by sympathetic system activation.

The present study had several limitations. The relatively small size of the sample studied limited the statistical power of the analysis. Nevertheless, the influence of several major determinants of LV mass, such as body build or BP, is detectable in even smaller population samples. Echocardiography, when used for LV mass assessment, shows limited reproducibility; however, subjects with conditions that compromise assessment by echocardiography (overweight, asymmetric septal hypertrophy, wall asynergy) were excluded from the study. Furthermore, as echocardiography had been performed some considerable time before TH gene polymorphism

assessment, the observers could not have known the results of DNA analysis. Nevertheless, this study should be regarded as a pilot study in the investigation of the effect of TH polymorphisms on the cardiovascular system.

To our knowledge, the present study is the first to demonstrate a significant association between Val-81-Met TH gene polymorphism and cardiac structure in a human population. TH appears to be a promising candidate gene for LV structure determination. The finding requires confirmation by a larger population study and in patients with pathological conditions such as hypertension and heart failure. Further research will also be needed to elucidate the mechanism by which the TH polymorphisms influence cardiac structure. □

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