ORIGINAL ARTICLE

Genetic determination of an endothelial function and the size of the heart sections in juvenile hypertensives

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Summary
Arterial hypertension is a polygenic disease and about 50 candidate genes have been analysed. We followed the size of the heart sections and the endothelial function in juvenile hypertensives according to the polymorphisms in the genes for angiotensin converting enzyme (I/D), endothelin 1 (Lys198/Asn) and endothelin 1 converting enzyme (Thr341/Ile).

We observed 44 juvenile hypertensives and 94 controls of the same age. An echocardiographic examination was carried out during standard examinations. The endothelial function was analysed with the use of ultrasound with high acuity after revoking a reactive hyperaemia. The genotypisations were performed using the polymerase chain reaction and restriction analysis. Statistical analyses were carried out using the BMDP statistical software.

There were no differences between controls and hypertensives in alleles frequency. The hypertensives differed from the control group in the endothelial function (statistically significant). The hypertensives homozygotes D/D and the heterozygotes Lys198/Asn had a larger left ventricle (statistically significant); the hypertensives with the Thr341/Ile had a larger left ventricle septum (statistically significant) and back wall of the left ventricle (statistically significant) than the controls with the same genotype.

We concluded that the genes for ACE, endothelin 1 and endothelin 1 converting enzyme have an influence on the size of the heart sections and on the endothelial function of the juvenile hypertensives.

Keywords: juvenile hypertension – polymorphisms – angiotensin converting enzyme – endothelin-1 – endothelin-1 converting enzyme

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Abbreviations
HT, hypertensives; C, controls; SBP, systolic blood pressure; DBP, diastolic blood pressure; ED, endothelial (dys)function; I, insertion; D, deletion; LV, left ventricle; ACE, angiotensin converting enzyme; E-1, endothelin-1; ECE, endothelin converting enzyme; PCR, polymerase chain reaction; T, thymin; G, guanine; C, cytosine; A, adenin.

INTRODUCTION

Genetic predispositions and enviromental factors play an important role in the development of cardiovascular diseases. The genetic outlook shows essential arterial hypertension as a polygenic disease and about 50 genes are being intensively focused on. The genes for angiotensin converting enzyme (ACE) (Hubáček and Poledne 1999), endothelin-1 (E-1) and endothelin-1 converting enzyme (E-1CE) belong to a group which has been intensively analysed (Zicha and Kuneš 1999, Adámková et al. 2002).

In the angiotensin converting enzyme gene, the insertion/deletion (I/D) polymorphism is the one most often analysed. The deletion allele (287 missing nucleotides in intron 16) of this gene is described as disadvantageous.

I/D polymorphism influences very distinctly the ACE level in blood, but not the blood pressure itself. The deletion allele was repeatedly found to be more common in patients after myocardial infarction (IM), though it was not proved that it would have been a risk factor for a repeated IM or a concomitant death. Similarly the D/D genotype was found with higher frequency in the patients suffering from an ischemic heart disease or a dilatation cardiomyopathy (Cambien et al. 1992, Marian et al. 1993, Raynolds et al. 1993, Hubáček and Poledne 1999, Hubáček et al. 2000, Adámková et al. 2001).

Edothelin-1 (E-1) is a potent paracrine vasoconstrictor peptide that acts as a modulator of a vasomotor tone, cell proliferation and vascular remodelling (Gulati et al. 1998).

The relationship between Lys198/Asn polymorphism and blood pressure has been described for pregnant women and obese people. The presence of Asn198 has been associated with higher blood pressure in many studies (Tiret et al. 1999, Cracowski et al. 1999, Vasku et al. 2000, Asai et al. 2001, Barden et al. 2001, Lajemi et al. 2001).

Endothelin-1 converting enzyme is a metaloproteinase enzyme that generates endothelin out of its precursor and thus it plays a determinant role in the regulation of the endotheline system. The only polymorphism changing the amino acid is Thr341/Ile and it has not yet been monitored in relation to blood pressure (Chackalamannil et al. 1996, Turnet et al. 1998, Schneider, 2002).

It is known that atherosclerosis risk factors often change the vessel wall function before atherosclerotic changes occur. The part of the vessel wall mainly affected is the endothelium, so we speak about changes of the endothelial function. Our purpose is to describe early changes in the endothelial function as early as possible. Longterm, insufficiently recompensed arterial hypertension hastens the growth of some parts of the heart's left ventricle (Widimsky 2002).

The distension of the septum, the back wall of the left ventricle and hypertrophy of the left ventricle are easily detected with the use of the transthoracle echocardiography and they both represent higher risks for the patient (Cracowski et al. 1999, Artinano and Gonzales 1999). Our target was to elicit the eventual differences in the extension of the left heart ventricle and the endothelial function in the juvenile hypertensives and the controls under different genetic determinations.

MATERIALS AND METHODS

The individuals analysed

We observed 44 juvenile hypertensives (aged 23.7 ± 3.17 years, 40 men, 4 women) and 94 control individuals of the same age (23.9 ± 3.21 years, 54 men, 40 women). All the observed individuals had had no antihypertensive therapy. Blood pressure was measured with the use of a mercury sphygmomanometre after 10 minutes of peace, while sitting down, on the left arm. The echocardiographic examination was carried out during a standard service in an echocardiographic laboratory. The size of the heart sections was measured in the standard projections and modes.

The endothelial function was analysed in the right brachial artery (Piťha et al. 2001). The evaluation of the dilatation was carried out with ultrasound with a high acuity after revoking a reactive hyperaemia.

An appropriately sized blood pressure cuff, attached to an automated oscillometric device (Boso Oscillomat, Bosch+Sohn, Jungingen, Germany) was placed on the left upper arm over the brachial artery. The individuals to be examined were kept at rest in the supine position for 10 minutes. At the end of this resting period, two blood pressure readings were taken using the automated oscillometric device.

Another blood pressure cuff, attached to a conventional mercury sphygmomanometre, was placed on the right forearm below the bend in the elbow and was inflated for 4 minutes to a pressure 60 mm greater than the mean of the two systolic blood pressure readings measured previously by the automated oscillometric device in the left brachial artery.

The endothelial function of the brachial artery was studied using high-resolution ultrasound. The diameter of the brachial artery was measured on B-mode ultrasound images using a linear-array transducer (median frequency, 7MHz), and an Acusson 128 XP/4 ultrasound system (Mountain View, California, USA). Scans were acquired with

the individual at rest and for 2 minutes during post tourniquet reactive hyperemia (to induce endothelium-dependent dilatation). Image analysis was performed off-line using a PC (Image Pro-Plus software, Media Cybernetics, Silver Springs, Maryland, USA). The measurements of the brachial artery were performed at the end of diastole (representing maximum dilatation) at 3 seconds intervals 10 times at rest, and at 3 seconds intervals 21 times immediately after the cuff had been deflated.

The primary measure of the analysis was the relative change in the mean arterial diameter, calculated as follows:

Percentage dilatation={(maximum diameter–baseline diameter)/(baseline diameter)}×100

Where maximum diameter is the mean of the 10 largest mean arterial diameters observed after cuff deflation, and baseline diameter is the mean of 10 baseline arterial diameters. All brachial studies and the computer assisted analyses were performed by the same physician blinded to records and all the data.

Genetic analysis
DNA was isolated using the standard method (Miller et al. 1998). The analysis of the ACE polymorphism was performed with the polymerase chain reaction (PCR) following the previously described methods (Rigat et al. 1993, Yoshida et al. 1996).

The following oligonucleotides were used for the analysis of the Lys198/Asn polymorphism in the endothelin-1 gene by PCR: E1F 5´ TCT TTT CAT GAT CCC AAG CTG AAA GGC GA and E1R 5´ GCC CCG AAG GTC TGT CAC CAA TGT GC.

For the endothelin converting enzyme Thr341/Ile polymorphism the oligonucleotides sequences were as follows: ECEF 5´ TAG AGC CCT GGG CTG TGA GGA GGA GC and ECER 5´ CTT ACC ATC TGT CGG TGG TGT TGA TG.

PCR were performed in both these cases in 25 µl of reaction mixture containing: 50 mmol/l KCl, 10 mmol/l Tris (pH 8.3), 1.75 mmol/l $MgCl_2$, 0.2 mmol/l dNTP, 0.5 U Taq DNA polymerase and 200 pmol/l of oligonucleotides under the following conditions: initial denaturation 96°C/4 min, and 35 cycles (94°C/15 sec, 69°C/30 sec, 72°C/30 sec). The last amplification step was prolonged to 5 minutes. 10 µl of PCR products were cut overnight by 5 U of the restriction enzyme Sau3AI (New England Biolab). The enzyme was used for detection of both polymorphisms. Alleles were visualised after the electrophoresis in the 3% agarose gel using ethidium bromide.

Statistical analysis
The statistical evaluation was carried out using the statistical software BMDP PC 90, supplemented by the Bonferroni method of mutiple comparison. The Two Way Analysis of Variance (ANOVA), the rank Mann-Whitney test, the Chí-square test in the contingentive charts and the Fisher exact test were carried out as was also a calculation of the correlation coefficient and linear regression. All tests were performed at the significance level $2\alpha = 0.05$.

RESULTS

Description of the analysed groups
The hypertensives distinctly differed from the control group in the systolic and the diastolic blood pressure (statistically significant) and in the endothelial function of the brachial vessel (statistically significant, Table 1, Fig. 1).

Table 1. **The basic characteristics of the observed groups**

	HT (n = 44)	C (n = 94)	Statistics
SBP (mm Hg)	128.60 ± 9.48	113.59 ± 12.46	*
DBP (mm Hg)	81.08 ± 5.35	72.71± 6.09	*
HR (min)	74.25 ± 9.09	71.61 ± 6.86	*
BMI (kg/m²)	25.54 ± 3.94	22.83 ± 3.09	*
ED/initial (%)	4.26 ± 0.63 (n=39)	5.15 ± 0.82 (n=35)	*
ED/end (%)	4.35 ± 0.66 (n=39)	5.43 ± 0.81 (n=35)	*

HT, hypertensives; C, controls; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ED, endothelial function; * statistically significant.
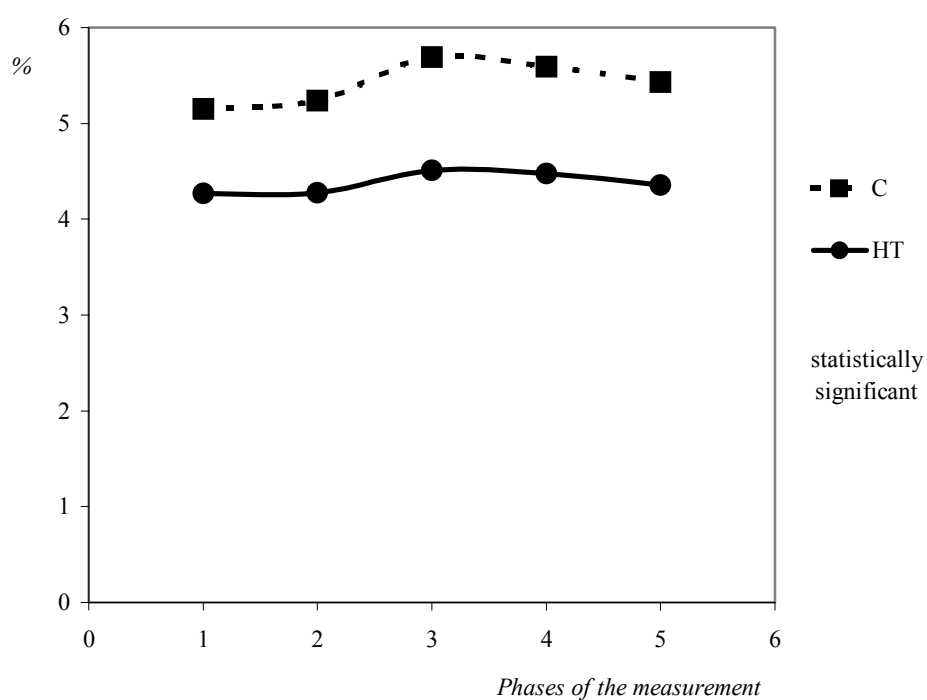
Fig.1 . **Endothelial function of the hypertensives** (HT) **and the controls** (C)

Table 2. **The frequency of the analysed polymorphisms (E-1, ECE, ACE) in the analysed groups**

Polymorphisms	HT (n = 44)	C (n = 94)	Statistics
E-1 Lys198/Lys	29 (67.4 %)	62 (66.0 %)	n.s.
E-1 Lys198/Asn	14 (32.6 %)	29 (30.8 %)	n.s.
E-1 Asn198/Asn	0	3 (3.2 %)	n.s.
E-1CE Thr341/Thr	41 (93.2 %)	80 (86.0 %)	n.s.
E-1CE Thr34/1Ile	3 (6.8 %)	13 (14.0 %)	n.s.
E-1CE Ile341/Ile	0	0	n.s.
	HT (n = 36)	C (n = 77)	Statistics
II	5 (12.2 %)	15 (16.9 %)	n.s.
ID	19 (54.8 %)	37 (50.8 %)	n.s.
DD	12 (33.0 %)	25 (32.3 %)	n.s.

I, insertion; D, deletion; Lys, lysin; Asn, asparagin; Thr, threonin; Ile, isoleucin; E-1, endothelin-1; ACE, angiotensin converting enzyme; ECE, endothelin converting enzyme; n.s., not significant; other symbols as in Table 1.

The frequency of allele polymorphisms of all three observed genes did not differ from the Hardy-Weinberg equilibrium. We did not find any significant differences between the control group and the juvenile hypertensives group (Table 2).

ACE I/D polymorphism
We found the left heart ventricle of D/D homozygotes hypertensives to be larger than that of the control individuals (statistically significant) with the same genotype. The I/I and I/D genotype carriers did not significantly differ in relation to the left ventricle.

Hypertensives I/I homozygotes had the biggest septum of the left ventricle: conversely we found the smallest left ventricle septum dimension in the control I/I homozygotes ($p < 0.01$, Table 3).

Table 3. **The size of the heart parts (mm) in ACE homozygotes D/D and I/**I

	HT D/D	C D/D	Statistics	HT I/I	C I/I	Statistics
N	12	25		5	15	
Left ventricle	51.9 ± 4.7	47.3 ± 4.6	*	50.0 ± 1.73	44.75 ± 2.63	n.s.
Septum of LV	8.50 ± 0.93	8.07 ± 1.38	n.s.	9.5 ± 0.58	7.25 ± 1.26	*
Back wall LV	8.75 ±1.67	7.93 ± 1.33	n.s.	8.75 ± 0.50	7.75 ± 1.71	n.s.

D, deletion; I, insertion; LV, left ventricle; other symbols as in Table 2.

Endothelin-1 Lys198/Asn polymorphism
A homozygote Asn198/Asn was found only in three individuals in the control group. In the group of the hypertensives this combination was not found.

The hypertensive heterozygotes Lys198/Asn had the larger left ventricle (n.s.) and left ventricle septum (statistically significant). The controls Lys198/Lys had the smallest left ventricle. The smallest left ventricle septum dimension was found in the Lys198/Asn controls (Table 4).

The difference in rating the quiescent endothelial dysfunction (ED) stage was significantly lower among the hypertensives Lys198/Lys and controls with the same genotype (4.2 ± 0.6% resp. 5.2 ± 0.9%, statistically significant) than among the carriers of Lys198/Asn (4.4 ± 0.5, resp. 5.0 ± 0.6%, statistically significant).

The greatest difference when rating other ED stages was found between the Lys198/Lys carriers; this is clear even in the last measurement (4.33 ± 0.71 %, resp. 5.56 ± 0.84 %, statistically significant).

Endothelin-1 convertig enzyme Thr341/Ile polymorphism
The hypertensives with the Thr341/Ile genotype had the larger left ventricle septum (9.66 ± 1.5 mm, vers. 7.2 ± 0.83 mm, statistically significant) and back wall of the left ventricle (9.0 ± 2.0 mm, resp. 7.2 ± 1.09 mm, statistically significant) than the controls. In contrast, these values did not differ for the hypertensives and the controls with a Thr341/Thr genotype. The endothelial function of the brachial artery mainly differed between the hypertensives and the controls of the Thr341/Thr genotype, (4.35 ± 0.61%, resp. 5.13 ± 0.85%, and phasis 4.45 ± 0.69, resp. 5.39 ± 0.80%, statistically significant).

Table 4. **The size of the heart parts** (mm) **and endothelin-1 Lys198/Asn polymorphism**

	Lys/Lys HT	Lys/Lys C	Statistics	Lys/Asn HT	Lys/Asn C	Statistics
N	29	62		14	29	
Left ventricle	49.16 ± 5.17	47.47 ± 3.59	n.s.	52.73 ± 4.58	48.40 ± 5.08	*
Septum of LV	8.40 ± 1.00	7.94 ± 1.35	*	8.81 ± 0.75	7.90 ± 1.28	*
Back wall of LV	8.48 ± 0.91	7.89 ± 1.45	*	8.63 ± 1.50	8.00 ± 1.49	n.s.

Symbols as in Table 1 and 2.

DISCUSSION

Essential arterial hypertension is a polygenic disease, the genetic predisposition to which is the result of the parallel action of many polymorphisms of various genes. Moreover, the unfavourable influence of environmental factors (e.g., diet, physical activity) may lead in individuals with the same combinations of genotypes to various manifestations and to varying seriousness of their disease (Adámková 2001).

We have analysed three variants in three different genes and their effects on the heart's sizes.

The frequency of alleles and genotypes of I/D polymorphism in the gene for ACE does not significantly differ in the observed groups where we found 29% of D/D, 50% of I/D and 22% of I/I and is practically equal to the previously published observations (Hubáček et al. 1999). The genotype frequencies of the E-1 polymorphism are congruent with the former findings from literature (Tiret et al. 1999, Cracowski et al. 1999, Vasku et al. 2000, Asai et al. 2001, Barden et al. 2001, Lajemi et al. 2001) and no data have been published so far concerning the allele frequencies of the The341/Ile variant in ECE.

Our results show that untreated juvenile ACE D/D homozygote hypertensives have a larger left ventricle than the controls. The extension of the left ventricle septum was the largest in the hypertensives with I/I combination. Thought the results in the literature are not unambiguous, the D/D homozygosity is considered as a disadvantage, but as is obvious from our results, it is possible that the homozygote I/I combination is favourable only to those individuals who are not under a higher risk of development of arterial hypertension due to some other, still unknown, factors.

Endothelin is a potent long-acting vasoconstrictor agent. The process of endothelin-1 biosynthesis is a slowly responding system that mediates chronic vasoconstrictor responses and resistance changes. Endothelin may be implicated in hypertension (in rats), and also has a potent mitogenic effect on the vascular smooth muscle cells, the glomerular mesangial cells and the cardiocytes.

In our group we found the largest left ventricle size and left ventricle septum size in hypertensive carriers of endothelin-1 Lys198/Asn genotype. Lajemi et al. (2001) observed C1363/T polymorphism in the same gene and it seems to be possible that the endothelin system may be responsible for the development of the cardial remodeling for people suffering from a serious form of heart malfunction but not for the lighter forms of arterial hypertension (Lajemi et al. 2001, Mpio et al. 1999). Further, they did not find any association between the 138I/D polymorphism for the E-1 gene and the left ventricle and the dimensions of the radial arteria. In our hypertensives, Lys198/Lys homozygotes had the lowest values of the endothelial function in contrast to the controls of the same genotype (statistically significant). No study has been published so far that has concentrated on an evaluation of the endothelial function for untreated juvenile hypertensives.

The frequency of Thr341/Ile heterozygotes (6.8% in juvenile hypertensives and 14% in controls) detected by us is so low that it makes it possible to interpret the results and shows a relatively rare appearance of Ile341 allele in the Czech population. We did not detect an Ile341/Ile homozygote. Heterozygote Thr341/Ile hypertensives had the largest left ventricle septum as well as thickness of the left ventricle back wall. The controls with the same genotype had the smallest left ventricle septum and also the left ventricle back wall.

The results of our survey show that the polymorphisms in the genes for ACE (I/D), endothelin-1 (Lys198/Asn) and yet unobserved polymorphism in endothelin-1 converting enzyme (Thr341/Ile), influence the endothelial function and the size of the heart sections of juvenile hypertensives.

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