

# The ScaI Gene Polymorphism of the Atrial Natriuretic Factor and Essential Arterial Hypertension in Childhood

Ruda Zorc-Pleskovič<sup>1</sup>, Matjaž Bidovec<sup>2</sup>, Dejan Bregar<sup>1</sup>,  
Aleksandra Milutinović<sup>1</sup>, Rifet Terzić<sup>3</sup> and Nataša Teran<sup>4</sup>

<sup>1</sup> Institute of Histology and Embryology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

<sup>2</sup> Department of Nephrology, Pediatric clinic, Medical Centre Ljubljana, Ljubljana, Slovenia

<sup>3</sup> Department of Biology and Human Genetics, Medical School Tuzla, Tuzla, Bosnia and Herzegovina

<sup>4</sup> Division of Medical Genetics, Department of Obstetrics and Gynecology, Medical Centre Ljubljana, Ljubljana, Slovenia

## ABSTRACT

*In order to investigate the contribution of the atrial natriuretic factor (ANF) gene in pathogenesis of essential arterial hypertension (EAH), we analyzed the ScaI gene polymorphism of the ANF gene in a group of children with EAH. Fifty-eight children, aged 8–19 years, with the diagnosis of EAH were included in the association study and were compared to 57 subjects with normal blood pressure (the control group). Arterial hypertension was defined as systolic/diastolic blood pressure higher than the 95<sup>th</sup> age-gender-height percentile of the adopted reference values. We failed to demonstrate an association between the ScaI ANF gene polymorphism and EAH in childhood (OR=2; 95% CI 0.9–4.2; p=0.07), however, we provided evidence of an interaction between the ScaI ANF gene polymorphism and obesity defined as BMI over the 85<sup>th</sup> percentile (OR=13.1; 95% CI 1.6–106; p<0.001).*

**Key words:** atrial natriuretic factor, essential arterial hypertension, childhood, ScaI gene polymorphism of the atrial natriuretic factor

## Introduction

Several candidate genes for multifactorial cardiovascular disorders have been tested in the general population as well as in diabetic patients in Slavic populations<sup>1–11</sup>. Essential arterial hypertension (EAH) is a complex disease influenced by various genetic and environmental factors<sup>12</sup>. Between 1% and 3 % of children are diagnosed with EAH<sup>12</sup>. Due to early expression of EAH in children, it could be speculated that genetic factors play a more important role than environmental factors in the development of hypertension<sup>12–16</sup>. Several complex physiological systems affect blood pressure, including atrial natriuretic factor (ANF)<sup>12,18,19</sup>.

The ANF has several effects, which might influence blood pressure, such as suppression of the renin activity, inhibition of the synthesis and release of aldosterone, vasodilatation, diuresis and natriuresis<sup>18,20</sup>. The ANF is another important candidate gene for arterial hypertension in childhood; genetic variants of the ANF could influence blood pressure. So far, there has not been an association study about the ScaI gene polymorphism of ANF in Caucasians with EAH; however, there have been one report for African Americans and one for the Japanese with EAH published<sup>13,21</sup>.

In this case-control association study we analyzed the contribution of genetic variability of the ANF to the predisposition for EAH in childhood. For this purpose we analyzed the ScaI gene polymorphism of ANF in a group of Caucasian children with EAH.

## Materials and Methods

Fifty-eight patients with essential arterial hypertension, aged 8–19 years, and 57 subjects in the control group were enrolled in the study. Arterial hypertension was defined as systolic/diastolic BP (blo-

od pressure) measurements higher than the 95<sup>th</sup> age-gender-height percentile of the adopted reference BP values<sup>22</sup>. To exclude the cases of white-coat hypertension, ambulatory blood pressure monitor was used<sup>23</sup>. In our study, the values considered »normal« for both systolic and diastolic pressure were based on data obtained from the multicentric European study performed on healthy children and adolescents<sup>23</sup>. The device was adjusted for measurements every 20 minutes during the daytime (from 7 a.m. to 9 p.m.) and 30 minutes during the nighttime. Children with secondary hypertension were excluded from the study. The patients and control subjects came from independent families from the same region, and they were all Slovenians. The data and blood samples of age-matched controls were obtained from pediatricians. The national medical ethics committee approved the study protocol. After informed consent was obtained from the patients' parents and control subjects, a detailed interview was made. Obesity was defined when body mass index (BMI) was greater than the 85<sup>th</sup> percentile per age per sex<sup>24</sup>.

The PCR reaction for ScaI ANF gene polymorphism was carried out in a 15- $\mu$ l reaction volume containing 0.17 mM dNTP (Gibco, UK), 10 mM TRIS HCl buffer, 1 mM MgCl<sub>2</sub>, 0.2  $\mu$ M of each primer (5'-GGC ACA CTC ATA CAT GAA GCT GAC T-3', 5'-GCA GTC TGT CCC TAG GCC CA-3' for ScaI ANF gene polymorphism), 500 ng of genomic DNA and 0.75 units of Taq DNA polymerase (Gibco, UK). DNA was amplified for 30 cycles with denaturation at 94 °C for 1 min, annealing at 61 °C for 1 minute, and primer extension at 72 °C for 40 seconds. 8  $\mu$ l of the PCR product was digested overnight at 37 °C with 10 units of ScaI, and the fragments were then separated on 8 % polyacrylamide gels<sup>25</sup>. The genotype polymorphism was detected as either A2A2 (presence of restriction site on both

alleles), or A1A2 (absence of a restriction site on one allele), or A1A1 (absence of restriction site on both alleles).

Differences in mean values were analyzed by Student's *t*-test and presented as means ± standard deviation (SD). Chi-square ( $\chi^2$ ) test was used to compare discrete variables. Genotypic odds ratios (OR) for EAH with 95% confidence intervals (CI) were calculated with two-tailed *p* values. Statistical analyses were performed using the SPSS program for Windows 98, version 11 (SPSS Inc., Illinois).

**Results**

Characteristics of the patients and control subjects are listed in Table 1. The ANF genotype distribution in cases and controls (Table 2) were compatible with Hardy-Weinberg expectations (ANF: cases *p*=0.2,  $\chi^2$ =1.66; ANF: controls *p*=0.524,  $\chi^2$ =3.763).

Univariate analysis (Table 3) showed a trend towards the association between the A1A1 genotype of the ScaI ANF gene polymorphism and EAH in childhood (OR=2; 95 % CI 0.9–4.2; *p*=0.07). Additionally, an interactive effect on the risk of EAH was found between obesity and the A1A1 genotype of the ScaI ANF polymorphism (OR=13.1; 95 % CI 1.6–106; *p*<0.001) (Table 3).

**Discussion**

In the study we failed to demonstrate an association between the ScaI ANF gene polymorphism and EAH in childhood. Lack of association between the ScaI ANF gene polymorphism and EAH is in accordance with two other studies in adults of different origin, Japanese and African American<sup>13,21</sup>. Interestingly, however, there are great differences among populations: A2 allele was very rare in the Japanese population (in 1.6 % of cases and in 4.6 % of controls), higher in our study in Caucasians (in 25.8 % of cases and in 36 % of controls), and even higher in African Americans (in 58.3 % of cases and in 61.4 % of controls)<sup>13,21</sup>. Moreover, case-control association studies are prone to type II errors (i.e., failing to reject the null hypothesis – that there is no difference in allelic distributions between the two groups – when it is false). One way to circumvent this problem is to increase the study sample.

In this study, however, we found a trend (*p*=0.07) towards an association between the A1A1 genotype of the ScaI gene polymorphism and childhood EAH. Although statistical significance did not reach *p*<0.05, the result obtain could indicate the involvement of the polymorphism as a risk factor in EAH. However,

**TABLE 1**  
CHARACTERISTICS OF PATIENTS WITH EAH AND CONTROLS

Characteristics	Patients	Controls	<i>p</i> value
Number	58	57	ns
Age (years)	14.7 ± 2.3	13.9 ± 2.5	ns
Height (cm)	169.3 ± 14.4	154.5 ± 39.2	<0.001
Weight (kg)	74.3 ± 19.3	50.5 ± 18.2	<0.001
BMI (kg/m <sup>2</sup> )	25.8 ± 5.5	19.7 ± 2.9	<0.001
Systolic blood pressure (mmHg)	148.2 ± 22.8	110 ± 23.3	<0.001
Diastolic blood pressure (mmHg)	86.7 ± 17.6	67.5 ± 14.7	<0.001

Values are means ± SD

**TABLE 2**  
DISTRIBUTION OF SCAI-ANF GENOTYPES AND ALLELES AMONG EAH PATIENTS AND CONTROLS

Variable	Patients N (%)	Controls N (%)
A1A1 genotype <sup>1</sup>	30 (51.7 %)	20 (35.1 %)
A1A2 genotype <sup>2</sup>	26 (44.8 %)	33 (57.9 %)
A2A2 genotype <sup>3</sup>	2 (3.4 %)	4 (7.0 %)
Allele distribution <sup>4</sup>		
A1 allele	86 (74.1 %)	73 (74.0 %)
A2 allele	30 (25.9 %)	41 (36.0 %)

<sup>1</sup> A1A1 genotype – absence of restriction site on both alleles<sup>2</sup> A1A2 genotype – absence of a restriction site on one allele<sup>3</sup> A2A2 genotype – presence of restriction site on both alleles<sup>4</sup> chi square=2.3, p=0.12**TABLE 3**  
INTERACTION BETWEEN THE SCAI GENE POLYMORPHISM OF THE ANF AND OBESITY  
IN CHILDREN WITH EAH AND CONTROLS

Variable	Patients N (%)	Controls N (%)	p	OR (95 % CI) <sup>1</sup>
A1A1 genotype <sup>2</sup>	30 (52)	20 (35)	0.07	2 (0.9–4.2)
BMI>85 <sup>th</sup> % <sup>3</sup>	35 (60)	8 (14)	<0.001	9.5 (3.8–23.9)
A1A1 genotype + BMI>85 <sup>th</sup> %	11 (19)	1 (2)	<0.001	13.1 (1.6–106)

<sup>1</sup> Odds ratio (95 % confidence interval)<sup>2</sup> ScaI gene polymorphism of the ANF<sup>3</sup> BMI was greater than 85<sup>th</sup> percentile per age per sex

the result should be interpreted cautiously because of a small sample.

Additionally, we provide evidence of an interaction between the ScaI ANF gene polymorphism and obesity defined as BMI over the 85<sup>th</sup> percentile. Children having the A1A1 genotype of the ScaI ANF polymorphism might be at a higher risk of increased blood pressure if they are obese. Based on our results and on some other studies it is reasonable to believe that the interaction between multiple genes rather than variants of a single gene underlie the genetic basis of EAH<sup>15,16</sup>. Sharma et al. (2000), who have systematically studied the entire human genome for major susceptibility genes for EAH, failed to demonstrate that a single region makes a large contribution to its origin.

They provide evidence that EAH is likely to be accounted for by several loci<sup>15</sup>. Future studies should therefore be oriented towards revealing a complex network of a variety of interacting genes, pathophysiological conditions (obesity, gender), and environmental factors.

In conclusion, we failed to demonstrate an association between the ScaI ANF gene polymorphism and EAH in childhood; however, we demonstrate evidence of interaction between the ScaI ANF gene polymorphism and obesity defined as BMI over the 85<sup>th</sup> percentile.

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*N. Teran*

*Division of Medical Genetics, Department of Obstetrics and Gynecology, Medical Centre Ljubljana, Slajmerjeva 3, Ljubljana 1000, Slovenia*

## **POLIMORFIZAM ScaI GENA ZA ATRIJSKI NATRIURETSKI FAKTOR I ESENCIJALNA ARTERIJSKA HIPERTENZIJA U DJETINJSTVU**

### **SAŽETAK**

U namjeri utvrđivanja uloge gena atrijski natriuretski faktor (ANF) u patogenezi esencijalne arterijske hipertenzije (EAH), ispitali smo ScaI polimorfizam ANF gena u djece s EAH. Ispitivanu skupinu čini 58 djece u dobi 8–19 godina, s dijagnozom EAH, a kontrolna skupina se sastoji od 57 ispitanika normalnog krvnog tlaka. Arterijska hipertenzija dijagnosticirana je kao sistoličko/dijastoličko mjerenje krvnog tlaka iznad 95. percentile referentnih vrijednosti, uzimajući u obzir i dob, spol, visinu. Nije dokazana povezanost ScaI ANF genskog polimorfizma i EAH u djece (OR=2; 95% CI 0.9–4.2; p=0.07), međutim, dokazali smo povezanost ScaI ANF genskog polimorfizma i pretilosti dijagnosticirane kao BMI iznad 85. percentile (OR=13.1; 95% CI 1.6–106; p<0.001).