Citosine-Adenine-Repeat Microsatellite of 11β-hydroxysteroid dehydrogenase 2 Gene in Hypertensive Children

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BACKGROUND
The impairment of 11β-hydroxysteroid dehydrogenase type 2 enzyme (11βHSD2) results in an inefficient conversion of cortisol to cortisone, which triggers hypertension. Cytosine-adenine repeat (CA repeat) microsatellite has been associated with low HSD11B2 gene expression.

AIM
To determine whether the CA-repeat length in intron 1 affect the serum cortisol to cortisone (F/E) ratio and/or blood pressure (BP) levels in pediatric subjects.

SUBJECTS AND METHODS
Eighty-one hypertensive (HT) and 117 normotensive (NT) subjects participated in this study. We measured BP levels, as well as the F and E and F/E ratio in morning sera and 12-hour urine samples. The length of CA repeats was determined through fragment analysis. We compared the allele distribution between the HT and NT groups, and the patients were dichotomized into groups with short alleles (S) (<21 CA repeats) or long alleles (L), and also in groups according genotype (allele combination: S/S and S/L + L/L).

RESULTS
We found no differences in the distribution of CA-repeat allelic length between the NT and HT groups ($P = 0.7807$), and there was no correlation between the CA-repeat allelic length and BP ($P = 0.1151$) levels or the serum F/E ratio ($P = 0.6778$). However, the serum F/E ratio was higher in the HT group than in the NT group ($P = 0.0251$). The serum F/E ratio was associated with systolic BP index independent of body mass index only in HT group.

CONCLUSIONS
The CA-repeat length did not influence BP levels or serum F/E ratios in pediatric subjects. However, the serum F/E ratio was associated with BP, suggesting a role of 11βHSD2 in mineralocorticoid hypertension.

Keywords: 11βHSD2; arterial hypertension; blood pressure; HSD11B2; hypertension; microsatellite region, short tandem repeat (STR), CA repeat.

doi:10.1093/ajh/hpv052

BACKGROUND

Impairment of the 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) enzyme results in an inefficient conversion of cortisol to cortisone (F/E) that determines the activation of the mineralocorticoid receptor (MR) by cortisol.[1–3] The MR has the same affinity for cortisol and aldosterone *in vitro*.[4–8] Activating the renal MR promotes the reabsorption of sodium, water and secondarily high blood pressure (BP).[9]

The expression of this enzyme has been reported in the following tissues: the renal distal, collecting tubule, brain, colon, placenta, pancreatic β cells, skin,[10] and human trophoblast, among others. The gene encoding 11βHSD2 is located in chromosome 16q22 (NG_016549.1; HUGO 5209); its promoter contains to NF1, GRE, and Sp1/Sp3 sites[11], it also includes 5 exons and possesses 2 microsatellite region of cytosine-adenine (CA) repeats; the first CA repeat is located in intron 1[12] (2,174 bp after the start of intron 1 and 1,900 bp before the end of intron 1), and the second is located 921 bp after the end of exon 5 (Figure 1).

DNA microsatellites are tandem repeat sequences formed by repeating units of 1–6 nucleotides. They are located in noncoding DNA sequences.[13] The effect of the length of CA repeats may depend on the number of repeats and also on

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Initially submitted December 19, 2014; date of first revision March 4, 2015; accepted for publication January 17, 2015; online publication April 23, 2015.

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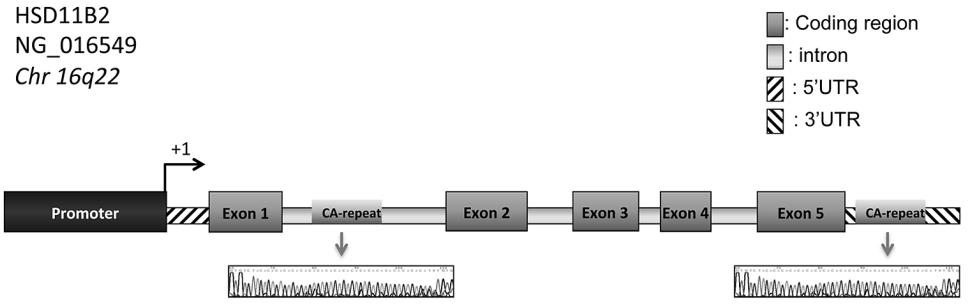

Figure 1. Gene structure of human 11BHSD2, which is located in the 16q22 chromosome and contains 5 exons. HSD11B2 gene promoter, the 5′UTR region on the left and right 3′UTR the region is shown. There are 2 tandem repeats of cytosine-adenine (2 microsatellite CA), the first is located in the intron 1 and the second is located in 3′UTR region. Abbreviation: CA, cytosine-adenine; UTR, untranslated region.

their location, relative to the gene. In general, microsatellites located near the gene promoter are more likely to influence the expression level of that gene. *In vitro* studies of the HSD11B2 gene based in a minigene construct that included the first intron (intron 1), showed that the minigene with 14 CA repeats demonstrated higher messenger RNA levels than the minigene with 23 repeats of CA.[14] However, this phenomenon did not occur in the second microsatellite[14] after exon 5.

Our group has previously reported that in adult with a longer microsatellite region in intron 1 of HSD11B2 gene, CA repeats are associated with higher serum cortisol to cortisone (F/E) ratios, which may favor hypertension.[15] Because all previous studies included either *in vitro* models[14] or adult patients,[16–20] we design and perform a study in pediatric and adolescent hypertensive (HT) patients to evaluate the influence of this microsatellite of CA repeats and their association with 11βHSD2 activity as measured by both serum and urine F/E ratio and subsequent BP.

SUBJECTS AND METHODS

Study design and sample description

A cross-sectional study was designed. We invited Chilean children and adolescents of both gender (4–15 years of age) to participate in this study. Patients who were using antihypertensive drugs, such as β-blockers, angiotensin-converting enzyme inhibitor, angiotensin II receptor blockers, diuretics, and spironolactone, were excluded. We recruited 198 subjects, who were classified and analyzed as HT (n = 81) and normotensive (NT, n = 117), according to the 4th Task Force guidelines (The 4th Task Force guideline define hypertension in children and adolescents as systolic BP (SBP) and/or diastolic BP (DBP) that is, on repeated measurement, at or above the 95th percentile (Table 1). We recruited from both primary school and HT outpatient programs associated to our Clinical Center. Subjects and patients were evaluated at the Pediatrics Endocrinology and Nephrology Reference Center of the Pontificia Universidad Católica de Chile. Height was measured using a wall-mounted Harpenden stadiometer Holtain (Crymych, Pembrokeshire, UK). Weight was assessed by bioelectrical impedance (Tanita Corporation of America, Arlington Heights, IL).[21] Trained nurses measured the BPs and heart rates of all subjects. Three consecutive measurements were taken from the right arm in 5-minute intervals with the patient in a seated position. Using an oscillometric method (Dinamap CARESCAPE V100; General Electric Healthcare; Medical Systems Information Technologies, Inc., Milwaukee, WI, USA), this procedure was performed according to the published recommendations.[16,21] In children, normotension was defined as an average SBP and/or DBP that was lower than 90th percentile for gender, age, and height. We calculated the BP index (SBP index (SBP_i) and DBP index (DBP_i)) to ensure that we could compare children from different gender, age, and stature percentiles. The BP index was determined using the observed BP/50th percentile BP level for gender, age, and height using the normal values reported.[16,21] All subjects with elevated BP were confirmed using the auscultatory method.[21] The HT and NT subjects had an ethnic origin of Spanish/white-Amerindian.[22]

Biochemical analysis

Fasting blood samples (drawn at 8:00–10:00 AM, from all subjects, who were in a sitting position, with at least a 15-minute rest) were obtained to measure the levels of the following biochemical parameters: sodium, potassium, cortisol, cortisone, plasma renin activity, and aldosterone; were measured as previously described.[21] At the same time, we collected 12-hour nocturnal urine (between 7:00 PM and 7:00 AM) samples. Total 12-hour urine volumes were measured, and 50 ml aliquots were stored to measure creatinine, sodium, and potassium levels. The serum, plasma, and urine samples were stored at −80 °C until analysis.

PCR amplification and determination of the length of CA-repeat microsatellites

Genomic DNA from the peripheral leukocytes of HT patients and NT controls was isolated and purified, according to the Lahiri technique.[23] PCR products in intron 1 of the HSD11B2 gene were obtained using the primers and the conditions previously described.[14,15,24] The primers flanked the CA-repeat microsatellite. The forward primer labeled with FAM fluorophore in 5′-end was designed and synthesized in IDT-DNA (San Diego, CA). All reactions were performed with 500 ng of genomic DNA in 25 µl of reaction volume containing GoTaq Colorless polymerase (Promega, Madison, WI). The sizes of the PCR products depended on the number of CA dimers repeated in the alleles (between 138 and 160 bp, corresponding to 13 and 24 CA repeats, respectively)

Table 1. Clinical and biochemical parameters of the patients and normal subjects

	Hypertensive (n = 81)	Normotensive (n = 117)	P
Clinical			
Gender (female/male; 98/100)	27/54	71/46	
Age (years)	12.06 (9.87–13.78)	11.06 (9.15–13.26)	0.1011
BMI_{SDS}	**1.73 (1.00–2.14)**	**1.04 (0.18–1.54)**	**<0.0001**
SBP_i	**1.18 (1.14–1.26)**	**1.03 (0.98–1.06)**	**<0.0001**
DBP_i	**1.22 (1.12–1.29)**	**1.08 (1.00–1.15)**	**<0.0001**
Blood biochemical			
Na (mEq/l)	141.0 (140.0–142.0)	141.0 (140.0–142.0)	0.7425
F (μg/dl)	**10.20 (6.95–14.35)**	**8.50 (6.50–11.25)**	**0.0065**
E (μg/dl)	3.30 (2.77–3.88)	3.18 (2.73–3.65)	0.0578
F/E ratio	**2.96 (2.49–4.09)**	**2.77 (2.19–3.42)**	**0.0251**
PA (ng/dl)	6.10 (4.10–8.55)	6.60 (3.50–8.70)	0.9406
PRA (ng/ml × h)	2.62 (1.76–3.90)	2.65 (1.74–3.87)	0.9809
ARR	2.30 (1.45–3.75)	2.30 (1.60–3.45)	0.7466
Urinary biochemical (nocturnal 12 hours)			
Na (mEq)	171.0 (104.0–218.0)	149.0 (108.0–207.5)	0.2422
Free F (μg)	11.40 (7.39–19.70)	11.70 (6.445–20.05)	0.9477
Free E (μg)	28.55 (2.15–37.10)	25.20 (10.40–34.00)	0.3372
Free F/E ratio	0.30 (0.21–0.43)	0.33 (0.23–0.42)	0.4203

The hypertensive children had higher serum cortisol levels, cortisol to cortisone (F/E) ratios, and BMI_{SDS} than normotensive children.

Abbreviations: BMI_{SDS}, body mass index (standard desviation); SBPi, systolic blood pressure index; DBPi, diastolic blood pressure index; Na, sodium; F, cortisol; E, cortisone; PA, plasma aldosterone; PRA, plasma renin activity; ARR, aldosterone to renin ratio. Statistical differences in biochemical parameters were higlighted in boldface.

from the subjects. Briefly, one aliquot of each PCR product was electrophoresed in 2% agarose gels, with molecular weight standards of 100 bp (Fermentas, Burlington, Ontario, Canada) to visualize amplicons. Another aliquot of each PCR product was used for DNA fragment analysis using capillary electrophoresis (ABI3730XLs; Applied Biosystems, Foster City, CA) in Macrogen (Seoul, Korea). We use a synthetic DNA of 152 bp to control the migration in electrophoresis, which corresponds to the same region amplified by PCR and is composed of 20 CA repeats (40 bp), alongside 92 bp upstream and 20 bp downstream. In all assays, the synthetic DNA control was amplified by PCR at the same time, with the subjects' samples. The capillary electrophoresis results were analyzed with the Peak Scanner software (Peak Scanner Software v1.0; Applied Biosystems), with the size standard GS400HD. Afterwards, we interpreted the electrophoretograms of these CA repeats in agreement to Arif *et al.*[25] study, to avoid and identify stutter-like alleles peaks.

The prevalence of allelic distributions and CA-repeat combinations were calculated. Short alleles were defined as fewer than 21 CA repeats (corresponding to <154 bp), and long alleles were identified as at least 21 CA repeats, according to previous reports.[14,15] Patients and subjects were classified according to their allelic length combinations (for each subject, the combination of both CA-repeat alleles in intron 1 of the HSD11B2 gene) to compare the distribution between HT and NT subjects (Supplementary Data). Then, for clinical and biochemical comparisons, the subjects were dichotomized into 2 groups, according to the length of their CA-repeat alleles: short/short (S/S), and both: short/long and long/long (S/L + L/L) together.

Data analysis

Normality was evaluated using the D'Agostino and Pearson omnibus test. All results were expressed as the median and interquartile range (Q1–Q3) and compared using the Mann–Whitney U-test and the Kruskal–Wallis test, with Dunn's multiple comparison tests. Alternatively, we used a t-test or a 1-way analysis of variance. The allele frequency was compared using χ^2 analysis. For all biochemical indicators with significant differences between the HT and NT groups, we evaluated correlations of these biochemical indicators with the BP index. Pearson's (r) or Spearman's (r_{sp}) correlations were used. To identify the associations between the genotypes and biochemical variables of cortisol and cortisone, which was seen as a continuous variable, a linear regression model was performed. The genotype variable we used was the sum total number of CA repeats in both alleles from each subject, adjusted by: age, gender, and BMI_{SDS}, and we checked for interactions of *a priori* selected variables and normality of residuals. Then, logistic regression analysis was performed, which reported crude and adjusted odds ratios for the association of HT with variables of interest. The influences of serum cortisol on the cortisone ratio and that of

BMI_{SDS} on BP were analyzed by multiple regression analysis. Statistical analysis was performed using the STATA and GraphPad Prism v.5 software (GraphPad, La Jolla, CA), and $P < 0.05$ was considered to be statistically significant.

Ethics

This study protocol followed the guidelines outlined in the Declaration of Helsinki and was approved by the Ethical Committee of the Faculty of Medicine, Pontificia Universidad Católica de Chile. The study and protocol were explained to all participants, and their parents provided signed informed consent. Subjects older than 7 years of age also provided signed assent before entering the study.

RESULTS

CA-repeat microsatellite of HSD11B2 is not associated with arterial BP levels and F/E ratios in children

Using microsatellite genotyping in both groups, we identified a diversity of CA-repeat lengths located in intron 1 of HSD11B2, ranging from 13 to 24 CA repeats (CA_{13} and CA_{24}, respectively). The most frequent allele was 20 CA repeat. The allelic frequency distribution of CA repeat was similar in HT and NT subjects (chi-squared = 1.75, DF = 4, $P = 0.78$, Table 2). We also found 29 allelic combinations. Only 2 of these combinations are most prevalent: $CA_{19/20}$ = 21% (5.55% HT and 15.65% NT) and $CA_{20/21}$ = 27.27% (10.10% HT and 17.17% NT), the other allelic combinations were presented in fewer than 6% of the subjects (Supplementary Data).

Association studies did not reveal any correlations between the length of CA-repeat allele in the whole group with SBP_i ($P = 0.11$, $n = 396$) or DBP_i, ($r_{sp} = 0.09$, $P = 0.06$, $n = 396$). Similar findings were seen in the HT group (SBP_i, $P = 0.08$; DBP_i, $P = 0.09$, $n = 162$ alleles) and the NT group (SBP_i, $r_{sp} = 0.051$, $P = 0.05$; DBP_i, $P = 0.72$, $n = 162$ alleles). In addition, we did not identify any correlations between the length of CA repeat and the serum F/E ratio (potentially claimed as 11BHSD2 activity) in the total group ($P = 0.68$, $n = 396$ alleles), HT group ($P = 0.3354$, $n = 162$), and NT group ($P = 0.7847$, $n = 234$). Moreover, length of alleles did not predict serum F/E ratio when we adjust by BMI_{SDS}, age, and gender ($R_2 = -0.008$, $P = 0.957$, $n = 198$). No significant differences were found in the SBP_i, DPB_i, and F/E ratio when the subjects were dichotomized according to the CA-repeat length (see Subjects and Methods and Table 3 for details).

Serum F/E ratio is associated with BP independently of BMI in HT children

In the whole group, we found that the serum F/E ratio is positively associated with SBP_i ($r_{sp} = 0.27$, $P < 0.0001$, $n = 198$ subjects) but not with DBP_i ($r_{sp} = 0.1381$, $P = 0.05$, $n = 198$) (Figure 2). HT subjects have higher serum F/E ratios than NT subjects (Table 1). We found significant higher levels of serum cortisol and a higher F/E ratio and BMI_{SDS} in the HT group compared with the NT group (Table 1). In the HT group, the serum F/E ratio correlated only with SBPi ($r_{sp} = 0.28$, $P = 0.01$, $n = 81$) and not with DBP_i ($r_{sp} = -0.04$, $P = 0.72$, $n = 81$) (graphics are in Figure 2). In the NT group, we found a positive correlation between the serum F/E ratio and $SBP_{i\ NT}$ ($r = 0.20$, $P = 0.03$, $n = 117$) and $DBP_{i\ NT}$ ($r = 0.19$, $P = 0.04$, $n = 117$) (Figure 2). No further associations were found between serum cortisol and SBPi ($P = 0.1061$, $n = 81$) and DBPi ($P = 0.5243$, $n = 117$) in either the HT or NT groups. Because we observed differences in the serum F/E ratio, we further correlated serum cortisol levels with SBP_i and DBP_i, but we did not find any correlations between serum cortisol and SBP_i (HT group, $P = 0.1061$, $n = 81$ and NT group, $P = 0.52$, $n = 117$) or DBP_i (HT group, $P = 0.25$, $n = 81$ and NT group, $P = 0.38$, $n = 117$).

In the HT group, stepwise multiple regression analysis showed that SBP_i but not DBP_i could be determined by F/E

Table 2. Allele frequency in patients and normal subjects

CA repeat	Total alleles number (%)	Number of alleles from HT subjects number (%)	Number of alleles from NT subjects number (%)
13	1 (0.003)	0	1 (0.004)
14	21 (0.053)	12 (0.074)	9 (0.038)
15	4 (0.010)	2 (0.012)	2 (0.009)
16	3 (0.008)	2 (0.012)	1 (0.004)
17	6 (0.015)	1 (0.006)	5 (0.021)
18	19 (0.048)	**7 (0.043)**	**12 (0.051)**
19	72 (0.182)	**24 (0.148)**	**48 (0.205)**
20	142 (0.359)	**60 (0.370)**	**82 (0.350)**
21	97 (0.245)	**39 (0.241)**	**58 (0.248)**
22	26 (0.066)	**11 (0.068)**	**15 (0.064)**
23	4 (0.010)	3 (0.019)	1 (0.004)
24	1 (0.003)	1 (0.006)	0
Total	396 (1)	162 (1)	234 (1)

The chi-squared results of hypertensive (HT) and normotensive (NT) subjects were calculated using allelic length combinations with ≥5% of the subjects in both groups (highlighted with boldface). Chi-squared = 1.755; DF = 4; $P = 0.7807$.

Table 3. Clinical and biochemical comparison between subjects with two short alleles and subjects with 1 or 2 long alleles

	S/S	S/L + L/L	*P*
N	87	111	
Long alleles (%)	0	57.7	-
Clinical			
Gender (female/male; 98/100)	41/46	57/54	Chi-squared = 0.3483, DF = 1, *P* = 0.5551
Age (years)	11.52 (9.71–13.65)	11.32 (8.81–13.35)	0.5248
BMI_{SDS}	1.21 (0.24–1.81)	1.34 (0.49–1.93)	0.5420
SBP_i	1.06 (1.01–1.15)	1.08 (1.01–1.17)	0.5721
DBP_i	1.11 (0.04–1.19)	1.14 (1.04–1.22)	0.3498
Hypertensive subjects (%)	36 (41.38)	45 (40.54)	Chi-squared = 0.01419, DF = 1, *P* = 0.9052
Blood biochemical			
Serum F (μg/dl)	9.50 (6.50–13.30)	8.80 (6.90–12.20)	0.7729
Serum E (μg/dl)	3.30 (2.64–3.86)	3.20 (2.80–3.66)	0.7313
Serum F/E ratio	2.85 (2.23–3.55)	2.86 (2.28–3.76)	0.9234
PA (ng/dl)	6.50 (4.10–8.90)	6.00 (3.40–8.50)	0.6453
PRA (ng/ml × h)	2.20 (1.40–3.80)	2.30 (1.60–3.40)	0.9382
ARR	2.77 (1.98–3.95)	2.47 (1.52–3.89)	0.2888
K (mEq/l)	4.30 (4.10–4.60)	4.30 (4.10–4.70)	0.5297
Urinary biochemical (nocturnal 12 hours)			
Na (mEq/l)	53.63 (35.70–79.55)	53.13 (37.63–85.64)	0.8056
FeNa	0.58 (0.40–0.80)	0.67 (0.47–0.88)	0.0460
Free F (μg)	5.02 (3.18–7.32)	3.87 (1.88–6.62)	0.0204
Free E (μg)	15.54 (10.54–21.81)	11.45 (7.61–17.25)	0.0088
Free F/E ratio	0.34 (0.24–0.44)	0.30 (0.22–0.42)	0.1900
K (mEq/mg Crea)	0.04 (0.03–0.06)	0.04 (0.03–0.06)	0.8493

The BP index was calculated using the BP/50th percentile level for gender, age, and stature with the normal values reported.

Abbreviations: ARR, aldosterone to renin ratio; BMI_{SDS}, body mass index; BP, blood pressure; DBP_i, diastolic blood pressure index; F/E ratio, cortisol to cortisone ratio; PA, plasma aldosterone; PRA, plasma renin activity; SBP_i, systolic blood pressure index.

ratio ($R^2 = 0.059$, $\beta = 0.243$, $P = 0.029$, $n = 81$), independent of BMI_{SDS}. Our results suggest that the F/E ratio could explain hypertension in at least 6% of HT subjects. By the other hand, in NT group, stepwise regression analysis showed that BMI_{SDS} can influence SBP_i ($R^2 = 0.072$, $\beta = 0.267$, $P = 0.004$, $n = 117$) and DBP_i ($R^2 = 0.039$, $\beta = 0.198$, $P = 0.032$, $n = 117$).

We compared HT patients and NT subjects, and we found no difference in the ratio of urine free cortisol to urine free cortisone (Table 1). Moreover, we did not find correlation between the urine free cortisol/urine free cortisone ratio and either SBP_i or DBP_i in the total group (NT + HT) (SBP_i $P = 0.0539$ and DBP_i $P = 0.8217$, respectively), NT group (SBP_i $P = 0.1146$ and DBP_i $P = 0.645$) and HT group (SBP_i $P = 0.2604$ and DBP_i $P = 0.4689$).

DISCUSSION

In the present study, we report that HT children have a higher serum F/E ratio than NT children (Table 1). We observed that the length of CA repeat in intron 1 of HSD11B2 did not influence either the serum F/E ratio or the BP in pediatric subjects. However, we observed that the serum F/E ratio is associated with SBP only in HT subjects independent of BMI_{SDS}, suggesting that 11BHSD2 activity could affect BP in children.

In our study, we found 12 alleles with different lengths, according to the number of CA repeats ranging from 13 to 24 CA repeats (CA_{13} to CA_{24}) (Table 2). A similar quantity of alleles was reported in both a Japanese and a Italian study, but in those studies, there were a different number of CA repeats between CA_{15} and CA_{25} and between CA_{14} and CA_{24}, respectively.[14,24] The most frequent alleles in our group were CA_{20} (35.9%); CA_{21} (24.1%) and CA_{19} (18.2%), unlike the Italian report, in which only the CA_{21} was the most frequent allele.[14] The most frequent combination in our group were $CA_{20/21}$ (24.69) and $CA_{19/20}$ (13.6%) (Supplementary Data).

When we compared the allelic distribution between the HT patients and NT subjects, we did not find any significant difference (Table 2), similar to a previous study of adult men by Mune *et al.*[24] Moreover, we did not find differences between CA-repeat length with clinical-biochemical

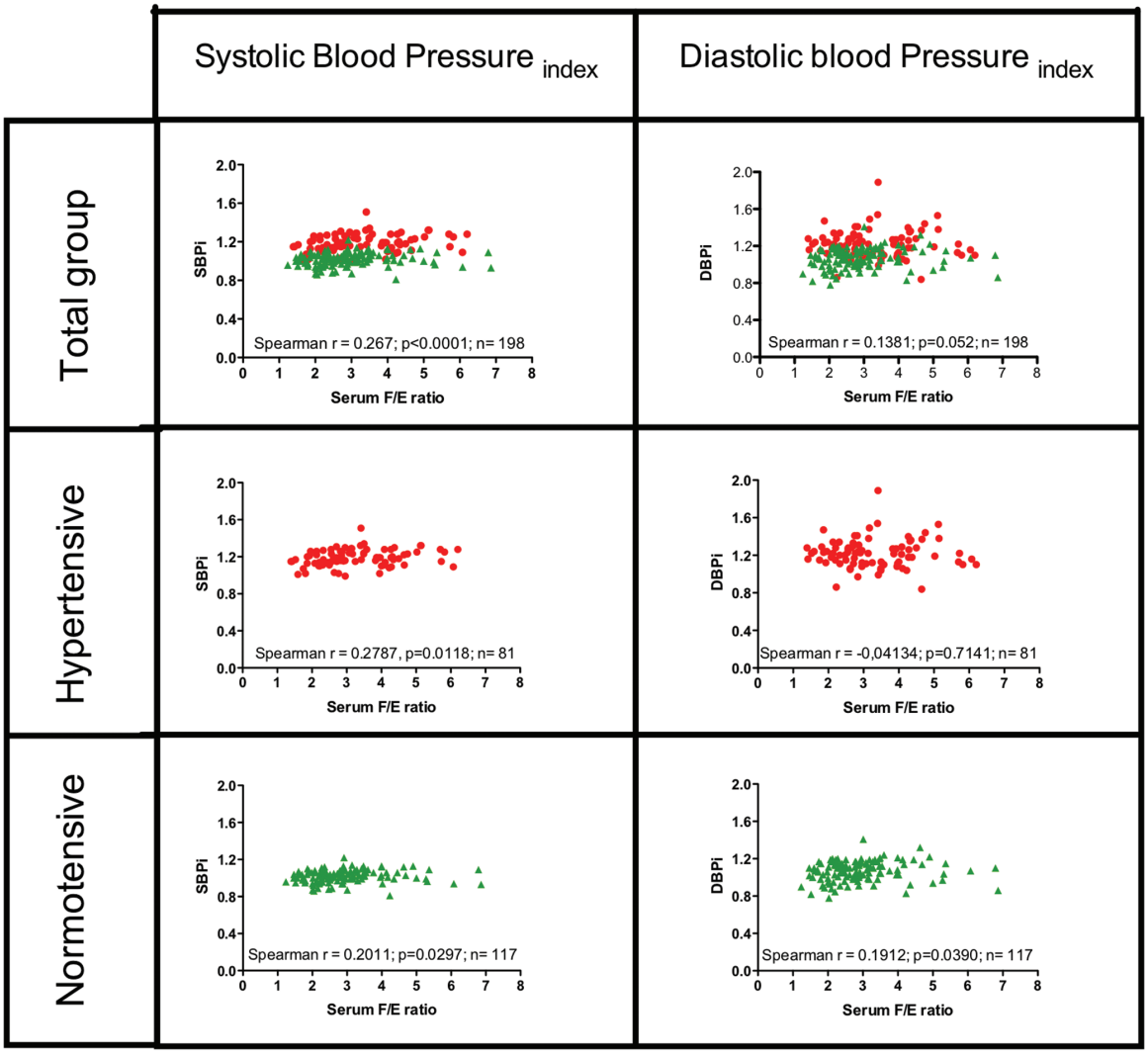

Figure 2. Correlation between the serum F/E ratio and blood pressure index. In hypertensive and normotensive groups we show a association between serum F/E ratio and SBPi. However, only in the hypertensive group, stepwise multiple regression analysis showed that SBPi but not DBPi could be determined by cortisol to cortisone ratio, independent of BMI_{SDS}. Abbreviations: BMI_{SDS}, body mass index (standard desviation); DBPi, diastolic blood pressure index; F/E ratio, cortisol to cortisone ratio; SBPi, systolic blood pressure index.

variables studied (i.e., BP, serum cortisol, serum cortisone, serum F/E ratio, plasma aldosterone, plasma renin activity, and aldosterone to renin activity ratio) (Table 3), suggesting that microsatellite in intron 1 of HSD11B2 is not supporting the development or progression of the HT condition. We evaluated the allelic and genotypic length in 81 HT children, however, a limitation of this study was the small number of HT, which could influence the statistical significance of genetic association between genotype and phenotype.

The serum F/E ratio was higher in HT children than in NT children; in addition, the F/E ratio but not cortisol was correlated with BP. After adjusting for BMI_{SDS}, in the HT patients, we observed that the SBP_i was retained as a dependent variable of the F/E ratio, suggesting that in HT patients, 11BHSD2 activity could affect BP. In cases of 11βHSD2 deficiency, cortisol is able to interact with the renal MR, inducing sodium and water reabsorption.[26] Moreover, it has recently been postulated that direct cortisol activation of the MR in nonepithelial cells, such as endothelial cells and smooth muscle cells, could lead to a pathophysiological process that induces vascular dysfunction, arterial stiffness and decreases nitric oxide bioavailability leading to vasoconstriction and hypertension.[27–29]

It has been proposed that renal activity of HSD11B2 can be measured by the urine free cortisol/urine free cortisone ratio,[30] but we did not find any difference between the HT and NT groups (Table 1). In addition, urine free cortisol/urine free cortisone ratio has not been associated with the BPi; thus, its predictive value is questionable in mineralocorticoid hypertension. We suggest that in an episodic peak in the morning, cortisol could increase but not enough to increase the 12-hour nocturnal urine collection, in which the nadir of Adrenocorticotropic Hormone (ACTH) occurs.[31] Thus, if any subtle 11βHSD2 impairment is occurring, it could be detected by the morning F/E ratio in serum when the circulating cortisol level is higher than urine F/E ratio.

Other authors have recently reported that this microsatellite influencing changes in blood glucose,[32] salt sensitivity, and fetal and maternal cortisol levels.[33] However, the molecular mechanisms that influence 11βHSD2 bioactivity are still unclear. Our results rule-out a possible effect of the length of the first microsatellite in HSD11B2 gene that affects serum cortisol and cortisone balance *in vivo*. Recently, we reported that serum F/E ratio correlated with age[34] and other possible mechanisms, such as the internal natural inhibitor of 11βHSD2 (e.g., the presence of Glicirretinic acid like factors (GALFs).[35–38] Finally, epigenetics modifications, such as methylated CpG islands,[39–43] could be occurring in HSD11B2 gene promoter, could also influencing the further 11βHSD2 expression.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

ACKNOWLEDGMENTS

We thank our patients and their families. This work was supported by the followings Chilean Grants FONDECYT (1130427, 1150437); FONDEF IDeA (CA12i10150); CORFO (13CTI-21526-P1); and the Millennium Institute of Immunology and Immunotherapy (MIII) P09/016-F (ICM).

DISCLOSURE

The authors declared no conflict of interest.

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