Frequency of Familial Hyperaldosteronism Type 1 in a Hypertensive Pediatric Population
Clinical and Biochemical Presentation

Marlene Aglony, Alejandro Martínez-Aguayo, Cristian A. Carvajal, Carmen Campino, Hernán García, Rodrigo Bancalari, Lilllian Bolte, Carolina Avalos, Carolina Loureiro, Pamela Trejo, Karin Brinkmann, Vinka Giadrosich, Verónica Meriq, Ana Rocha, Alejandra Avila, Viviana Perez, Andrea Inostroza, Carlos E. Fardella

See Editorial Commentary, pp 1053–1055

Abstract—Familial hyperaldosteronism type 1 is an autosomal dominant disorder attributed to a chimeric CYP11B1/CYP11B2 gene (CG). Its prevalence and manifestation in the pediatric population has not been established. We aimed to investigate the prevalence of familial hyperaldosteronism type 1 in Chilean hypertensive children and to describe their clinical and biochemical characteristics. We studied 130 untreated hypertensive children (4 to 16 years old). Blood samples for measuring plasma potassium, serum aldosterone, plasma renin activity, aldosterone/renin ratio, and DNA were collected. The detection of CG was performed using long-extension PCR. We found 4 (3.08%) of 130 children with CG who belonged to 4 unrelated families. The 4 patients with CG had very high aldosterone/renin ratio (49 to 242). In addition, we found 4 children and 5 adults who were affected among 21 first-degree relatives. Of the 8 affected children, 6 presented severe hypertension, 1 presented prehypertension, and 1 presented normotension. High serum aldosterone levels (>17.7 ng/dL) were detected in 6 of 8 subjects (range: 18.6 to 48.4 ng/dL) and suppressed plasma renin activity (≤0.5 ng/mL per hour) and high aldosterone/renin ratio (>10) in 8 of 8 children (range: 49 to 242). Hypokalemia was observed in only 1 of 8 children. We demonstrated that the prevalence of familial hyperaldosteronism type 1 in a pediatric hypertensive pediatric population was surprisingly high. We found a high variability in the clinical and biochemical characteristics of the affected patients, which suggests that familial hyperaldosteronism type 1 is a heterogeneous disease with a wide spectrum of presentations even within the same family group. (Hypertension. 2011; 57:1117-1121.)

Key Words: arterial hypertension ● aldosterone ● familial hyperaldosteronism ● glucocorticoid-remediable aldosteronism ● children

Familial hyperaldosteronism type I (FH-I; Online Mendelian Inheritance in Man, No. 103900), which is also known as glucocorticoid-remediable aldosteronism, is often characterized by severe hypertension, variable hyperaldosteronism, low plasma renin activity (PRA), normal or decreased serum potassium, and abnormal adrenal steroid production, including 18-oxocortisol and 18-hydroxycortisol, in adults.1

FH-I occurs because of an unequal crossing over of the genes that encode the steroid 11ß-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B1) enzymes, which results in a chimeric CYP11B1/CYP11B2 gene (CG) with aldosterone synthase activity that is regulated by plasma adrenocorticotropic hormone levels instead of angiotensin II; this results in an ectopic expression of aldosterone synthase in the zona fasciculate.3

FH-I is an autosomal dominant disorder, and different pedigrees exhibit different crossover patterns of the hybrid gene, which suggests that the mutations arose independently in each pedigree.4 In the hypertensive adult population, this monogenic form of aldosteronism is thought to account for only 0.5% to 1.0% of primary aldosteronism; however, the exact prevalence remains to be established. In the hypertensive pediatric population, this information is unknown.
The diagnosis in children has been obtained basically from a genetic screening of first-degree relatives in FH-I–affected kindred. An early diagnosis is clinically relevant because FH-I could be specifically treated by suppressing adrenocorticotropic hormone production with cortisol or synthetic glucocorticoids, such as dexamethasone.

In this report, we show the prevalence of FH-I in a select Chilean hypertensive pediatric population and describe their clinical, biochemical, and molecular characteristics, which highlight the variability of their clinical presentation.

Patients and Methods

Subjects

A cross-sectional study was design. Chilean hypertensive patients without evident of secondary cause (n=148) aged 4 to 16 years were recruited between July 2009 and April 2010 from pediatric nephrology and endocrinology units at Pontificia Universidad Católica de Chile. The former unit is one of the national tertiary referral centers for pediatric hypertension study. In addition, others collaborating investigators referred patients for this study.

All of the subjects underwent a complete physical examination that was performed by 2 pediatric endocrinologists (A.M.-A. and H.G.), 1 pediatric nephrologist (M.A.), and 4 pediatricians with training in pediatric endocrinology (C.A., L.B., R.B., and C.L.). The subject height was measured using a wall-mounted Harpenden stadiometer (Holtain), and their weight and total fat mass percentage were assessed by bioelectrical impedance (Tanita, Corporation of America, Arlington Heights, IL). The children with severe obesity (z score: >2.5) were excluded. Pubertal development was assessed according to the Marshall and Tanner method.

Trained nurses measured the blood pressure (BP) and heart rate of all of the subjects and their parents. Three measurements were obtained consecutively from the right arm at 5-minute intervals using an oscillometric method (Dinamap CARESCAPE V100, GE Health-care) with the patient in a seated position. This was performed with a cuff and bladder size that was adjusted to the upper-arm girth according to the published recommendations. The office BP of the children and their parents was classified according to the Fourth High Blood Pressure guidelines. Prehypertension in children was defined as an average systolic BP and/or diastolic BP greater than the 90th percentile but less than the 95th percentile for sex, age, and height. Stage 1 hypertension was designated as BP levels that ranged from the 95th percentile to 5 mm Hg above the 99th percentile. All of the elevated BP measurements were confirmed using the auscultatory method. We calculated the BP index so that we could compare children of different sexes, ages, and stature percentiles. The BP index was determined using the observed BP/50th percentile BP level for sex, age, and stature using the normal values reported and calculated as described previously.

Ambulatory BP monitoring measurements were performed in 2 subjects with the CG who did not have clinical hypertension. A validated oscillometric device (SpaceLabs model 90217 monitor, SpaceLabs, Inc) was used to measure the ambulatory BP. The appropriate cuff, which was chosen from the 3 available sizes, was attached to the nondominant arm. The accuracy and precision of the automated measurements performed in individual subjects by the oscillometric monitors were confirmed with a mercury sphygmomanometer at the beginning of the test period. The frequency of the automated readings and the data analysis were performed with standard parameters. The following parameters were calculated for each subject: (1) total number of BP readings; (2) average systolic BP, diastolic BP, and heart rate over the 24-hour awake and sleep periods; and (3) circadian BP and heart rate variability, which was estimated as the awake/sleep time ratio of the systolic and diastolic BPs and heart rate averages.

We measured the serum creatinine (Cr), calcium, urea, glucose, and a hepatic profile (alkaline phosphatase, bilirubin, alanine aminotransferase, aspartate aminotransferase, and albumin) in all of the subjects. We used this screening to exclude subjects with renal disease, diabetes mellitus, hepatic failure, and hypercalcemia. With the aim of reducing the errors associated with children with endogenous hypercortisolism, we excluded those subjects with a combined reduced linear growth or increased weight. These elements are highly sensitive at identifying subjects who are at risk of having endogenous hypercortisolism.

We selected 130 hypertensive patients diagnosed recently and before the patients started their hypertensive treatment. Those patients who were under treatment with drugs that might affect the renin-angiotensin-aldosterone system were excluded before the blood samples were drawn. We did not find patients with suspect hypercortisolism or renal disease. However, we excluded 18 patients (please see the online Data Supplement at http://hyper.ahajournals.org).

After an overnight fast, basal blood samples were obtained between 8:00 AM and 10:00 AM. Subjects assumed a sitting position for at least a 15-minute rest period. The blood samples to measure potassium, sodium, Cr, cortisol, serum aldosterone (SA), and PRA and to calculate the SA/PRA ratio (ARR) were obtained from an indwelling catheter that was positioned in an antecubital vein.

According to our recently reported reference values for the pediatric population, we consider high SA levels as values that are >17.7 ng/dL (491.7 pmol/L) with a suppressed PRA at cutoff of ≤0.5 ng/mL per hour (which was the third percentile in Chilean normotensive healthy pediatric population) and high ARR levels as values that are >10. However, in our Chilean hypertensive adult population, the ARR level is considered high when it exceeds 25. In addition, 12-hour nocturnal urine (between 7:00 PM and 7:00 AM) samples were collected. Total 12-hour urine volumes were measured, and aliquots were stored to measure the urinary free cortisol, Cr, sodium, and potassium. We also calculated the fractional excretion of sodium. Genomic DNA was also isolated from peripheral blood leukocytes. In all of the hypertensive subjects, we performed a genetic analysis of the chimeric CYP11B1/CYP11B2 gene using the long-extension PCR technique, and further confirmation was obtained by sequencing.

Hormonal and Biochemical Assays

The results were expressed in international system of units according to the instructions of the journal. However, for SA, we used nanograms per deciliter (and picomoles per liter in parentheses), and for PRA, we used nanograms per milliliter per hour instead of micrograms per liter per hour. SA and PRA were measured as described previously. The normal adult range was 1 to 16 ng/mL (27.8 to 444.5 pmol/L) for SA and 0.66 to 2.34 ng/mL per hour for PRA. The lower limit of the PRA assay was 0.1 ng/mL per hour.

Serum and urinary free cortisol and serum and urinary Cr were measured as described previously. Urinary free cortisol was normalized by Cr (urinary free cortisol/Cr), and kidney function was estimated using serum Cr levels.

Genetic Study

A genetic study was performed to determine the presence of FH-I. Genomic DNA was isolated from peripheral blood leukocytes using a lysis buffer and DNAzol (Invitrogen). Genetic analysis for FH-I was conducted using long-extension PCR of the CYP11B1/CYP11B2 gene, using a protocol that was described previously by our group. This technique uses 2 long-extension PCR amplification reactions in which 2 sense primers and 1 antisense primer is used sequentially and is carried out using a GeneAmp long-extension PCR kit (Applied Biosystems). Sequence analysis of the CYP11B2 and chimeric gene were performed in an ABprism-377 DNA Sequencer (Applied Biosystems) and then aligned with CLUSTAL W. Paternity study was performed if both parents are negative for CG (please see the online Data Supplement).

None of the study populations had a history of psychiatric illness such as a mood disorder or anxiety disorder prior to the present study.
Table 1. Clinical and Biochemical Characteristics of the Pediatric Hypertensive Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Median</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>12.2</td>
<td>10.0</td>
<td>13.8</td>
</tr>
<tr>
<td>Height, SDS</td>
<td>0.52</td>
<td>-0.15</td>
<td>1.13</td>
</tr>
<tr>
<td>BMI, percentile</td>
<td>95.3</td>
<td>85.2</td>
<td>98.3</td>
</tr>
<tr>
<td>Body fat mass, %</td>
<td>29.1</td>
<td>23.6</td>
<td>39.0</td>
</tr>
<tr>
<td>Abdominal circumference, cm</td>
<td>80</td>
<td>69</td>
<td>92.5</td>
</tr>
<tr>
<td>SBP index</td>
<td>1.19</td>
<td>1.14</td>
<td>1.26</td>
</tr>
<tr>
<td>DBP index</td>
<td>1.23</td>
<td>1.11</td>
<td>1.33</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA, ng/dL</td>
<td>6.0</td>
<td>3.4</td>
<td>8.9</td>
</tr>
<tr>
<td>PRA, ng/mL per h</td>
<td>2.2</td>
<td>1.4</td>
<td>3.5</td>
</tr>
<tr>
<td>ARR</td>
<td>2.5</td>
<td>1.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Serum cortisol, nmol/L</td>
<td>275.9</td>
<td>185.9</td>
<td>391.2</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>50.39</td>
<td>43.32</td>
<td>58.34</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>4.4</td>
<td>4.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td>141</td>
<td>140</td>
<td>142</td>
</tr>
<tr>
<td>UFF/Cr, nmol/mmol</td>
<td>5.21</td>
<td>3.31</td>
<td>7.39</td>
</tr>
<tr>
<td>FENa 12 h</td>
<td>0.67</td>
<td>0.45</td>
<td>0.92</td>
</tr>
</tbody>
</table>

n=130; women, 37.8%; BMI indicates body mass index; SBP index, systolic blood pressure index; DBP index, diastolic blood pressure index; FENa, fractional excretion of sodium; SDS, standard deviation score; SA, serum aldosterone; PRA, plasma renin activity; ARR, SA/PRA ratio; UFF, urinary free cortisol; Cr, creatinine. To convert from conventional units to international system of units, divide cortisol (nmol/L)/27.59 and creatinine (μmol/L)/88.4 to ng/dL; and UFF/Cr (nmol/mmol)/0.3138 to μg/g. To convert from conventional units to international system of units, multiply SA (ng/dL)×27.743 to pmol/L. To convert PRA from ng/ml per hour to μg/L per hour multiply by 1.

Data Analysis

Statistical analyses were performed using the SPSS 15.0 program for Windows (SPSS, Inc.). Results were expressed as median values (interquartile range: Q1 to Q3).

Ethics

The protocol was approved by the ethical committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile in accordance with the Helsinki Declaration. All of the parents signed informed consent forms, and subjects who were >7 years old provided their consent before entering the study.

Results

We studied 130 hypertensive children. SA levels >17.7 ng/dL (491.7 pmol/L) were observed in 9 (6.9%) of the 130 patients, PRA levels ≤0.5 ng/mL per hour were observed in 4 (3.1%) of the 130 patients, and ARR levels >10 were observed in 5 (3.9%) of the 130 patients. The clinical and biochemical characteristics are shown in Table 1.

The prevalence of the hybrid CYP11B1/CYP11B2 gene in our hypertensive population was 4 (3.1%) of the 130 from 4 unrelated families. The geographic origins of the families included Santiago de Chile (family 1), Punta Arenas (families 2 and 3), and San Antonio (family 4), and the ethnicity of all 4 of the subjects was Hispanic. The age range at the diagnosis of the hybrid CYP11B1/CYP11B2 gene was between 4 and 15 years. The 4 affected children had hypertension stage 2, high SA, low PRA, and high ARRs, as shown in Table 2.

A search for the chimeric CYP11B1/CYP11B2 gene was conducted in 21 first-degree relatives from these 4 families. Three of the 4 index case children had ≥1 parent with the chimeric CYP11B1/CYP11B2 gene, which confirmed the presence of FH-I disease. In family 1, 7 members belonging to 3 generations were studied; the father (F1:II-2) and the sister (F1:III-2) were also CG positive. In family 2, 6 members belonging to 2 generations were studied; the father (F2:II-3) and the brother (F2:III-1) were affected. In family 3, 4 members belonging to 2 generations were studied. In this family, the CG analysis was negative for both parents, and the paternity study confirmed the nonparental relationship between the index case and his father. In family 4, 6 members belonging to 2 generations were studied; the CG was present in his mother (F4:I-2) and other siblings (F4:II-2 and II-5). A total of 9 of the 21 first-degree relatives were affected, 4 children and 5 adults (Table 2). For pedigrees details of the 4 families please see the online Data Supplement. In summary, 8 children with the CG were studied.

Blood Pressure Characterization in the Hybrid CYP11B1/CYP11B2 Gene Group

Of the 8 children affected, the 4 index cases had stage 2 systolic/diastolic hypertension. The youngest child presented with ataxia and vomiting at the age of 4 years. Brain MRI demonstrated an infarction in the cerebellum. There were no apparent underlying diseases, including hematologic, cardiac, and vascular abnormalities. Of the other 2 hypertensive children, one had stage 2 systolic/diastolic hypertension and the
other had stage 2 systolic and stage 1 diastolic hypertension. A prehypertensive stage was observed in 1 of the 8 patients, and 1 child (6-year old) was normotensive. Of the 5 adults, 3 were hypertensive (2 had systodiastolic hypertension, 1 had stage 1 hypertension, and 1 had stage 2 hypertension; the third adult had stage 2 systolic hypertension and diastolic prehypertension). The other 2 adults had systolic prehypertension and normal diastolic values. The BP levels of each family member in the 4 families are shown in Table 2. Ambulatory BP monitoring was performed in 2 subjects who had the CG and did not show clinical hypertension. The first patient (F4:II-5) was a 6-year-old girl who was clinically normotensive and displayed sustained normotension with a conserved circadian pattern; the second patient (F4:II-1) was a 20-year-old woman who showed prehypertensive systolic pressure, and the ambulatory BP monitoring pattern showed altered, systodiastolic hypertension during the nighttime period with an absence of the nocturnal dip.

Biochemical Characteristics in the Hybrid CYP11B1/CYP11B2 Gene Group

At the time of genetic diagnosis of FH-I, only 1 child was hypokalemic (range: <2.0 to 3.4 mmol/L). The SA levels were >17.7 ng/dL (491.7 pmol/L) in 6 of the 8 children (range: 18.6 to 48.4 ng/dL; 513 to 1337 pmol/L). All of the children (8 of 8) had suppressed PRA and ARR that were >10 (range: 56.5 to 242). Two adults had hypokalemia (F2:II-2 and F2:III-1), one of which was severe (<2 mmol/L) and 3 adults showed suppressed PRA levels (F2:II-3, F2:III-1, and F4:II-1). Three adults (F1:II-2, F2:II-3, and F2:III-1) had high SA levels (range: 19.8 to 69.4 ng/dL; 550.0 to 1927.8 pmol/L). In 3 of the 5 studied adults (F1:II-2, F2:II-3, and F2:III-1), the ARR was >25 (range: 46.2 to 236.5).

Response to Antihypertensive Treatment in the Hybrid CYP11B1/CYP11B2 Gene Group

The children and adults with the hybrid CYP11B1/CYP11B2 gene and hypertension were treated with cortisol (10 to 12 mg/m² per day) or dexamethasone (0.25 mg/d), respectively. In 10 of 13 patients, the monotherapy normalized their BP and biochemical parameters, whereas in 3 of 13 patients, the monotherapy failed to maintain BP below the 90th percentile. In patients F1:III-1, F2:III-3, and F3:III-2, it was necessary to add spironolactone according to the following dose schedules: 25 mg/d (10 to 20 kg), 50 mg/d (21 to 40 kg), and 100 to 200 mg/d (adults). With dexamethasone (0.25 mg/d) and spironolactone (100 mg/d) treatment, patient F2:III-3 remained hypertensive with suppressed PRA, and so the dose of spironolactone was increased to 200 mg/d, which resulted in an improvement in his BP values and laboratory parameters.

Discussion

In this study we demonstrated a high prevalence of FH-I in a hypertensive pediatric population, especially in patients with ARR >10. We also reported a high variability in the clinical and biochemical features of the affected patients, which suggests that FH-I is a heterogeneous disease with a wide spectrum of presentation even within the same family group; this supports previous findings reported by Fallo et al.19

The genetic test demonstrated a prevalence of 3.08% (4 of 130) of the chimeric CYP11B1/CYP11B2 gene in this pediatric hypertensive population. This prevalence appears to be surprisingly high if we considered those reported in hypertensive adult population where the prevalence of PA is close to 10%, and <1% of that population is affected by an FH-I.5

All of the index cases had severe hypertension, and one had a stroke. However, in the group of all the patients affected with FH-I, we found a high variability in the BP from slightly elevated BPs to severe hypertension. Studies of the International Registry for Glucocorticoid-Remediable Aldosteronism in 20 subjects report that 80% of affected children are hypertensive at the time of diagnosis or will develop hypertension by 13 years of age; however, normal or borderline BPs even in adult populations do not rule out the diagnosis.6 The presence of severe combined systolic and diastolic BPs or isolated systolic hypertension suggests FH-I, and the patient should be tested for this defect. None of the subjects had isolated abnormal diastolic BP.19

The 4 index cases had high SA levels, low PRA levels, and high ARR values. However, when we considered the total number of children that were affected, we found a great variability in the SA levels, PRA levels, ARR, and potassium values. In the children in the present study, the hypokalemia was present only in 12.5%, which supports the findings of previous studies that showed a low prevalence of hypokalemia, which lacks sensitivity as a screening test for FH-I.20,21 We also found a suppressed PRA in 100% of the children, high SA levels in 75%, and the coexistence of hypokalemia, suppressed PRA, and high SA levels in only 12.5% of them. The ARR also showed high variability, but in this pediatric population, all of the children with ARR >10 were always associated with a CG, which suggests that, at least in this population, a genetic test to confirm/rule out FH-I should be performed. We did not perform a dexamethasone suppression test because of the high prevalence of false positives that were demonstrated previously with its use.22

All of our CG subjects normalized their BP values with specific therapy using either cortisol or dexamethasone with spironolactone. FH-I should be treated medically with a glucocorticoid to partially suppress the pituitary adrenocorticotropic hormone secretion. In general, the lowest dose of glucocorticoid that normalizes the BP should be used. Treatment with a glucocorticoid may not always normalize the BP, and the addition of a mineralocorticoid receptor antagonist should be considered in these cases.1 In addition, we suggest that cortisol should be used in the pediatric population; dexamethasone could be an alternative only after the linear growth has been completed.

Recently, we reported an elevation in endothelial inflammatory markers in patients with FH-I and normalization of these markers after glucocorticoid treatment.23 These results provide new insight about the possible deleterious effects of aldosterone on the endothelium. We hypothesized that subjects with FH-I and normal BP but high levels of endothelial inflammatory markers are at risk for the development of
cardiovascular diseases and should be treated with glucocorticoids. To our knowledge, the information about frequency of FH-I in the pediatric hypertensive population is scarce. Our study has the advantage that the frequency of FH-I was determined in the whole hypertensive pediatric population independent of PRA and SA concentration. Other studies are retrospectives, and FH-I prevalence has been studied only in those patients with high aldosterone/PRA ratio.

In summary, our main finding was the importance of considering the diagnosis of FH-I in the setting of children with high ARR, which may or may not be associated with severe hypertension. We found a high variability in the clinical and biochemical parameters in families with FH-I and even in affected children and adults from the same family group. We recommend a genetic test for hypertensive children with an ARR >10 that is independent of the severity of the hypertensive disease or the absolute PRA or SA levels.

Perspectives

High frequency of FH-I was found in this Chilean hypertensive pediatric population. This diagnosis was based on high ARRs (>10) and was confirmed by testing for the chimeric CYP11B1/B2 gene. Similar to adult populations, independent values of plasma K⁺, SA, and PRA are not good markers of FH-I. An early clinical and genetic diagnosis in pediatric patients with FH-I will improve their treatment and will reduce the morbidity and mortality that is associated with this hypertensive disease.

Acknowledgments

We are indebted to our patients and their families.

Sources of Funding

This work was supported by Chilean Grants FONDECYT 1100356 and 1070876, FONDEF D08I1087, NMII P08/077-F (ICM). A.M.-A. is a recipient of a grant from Becas-Chile (www.becaschile.cl) and from the Pontificia Universidad Católica de Chile. C.A.C. is a fellow of Comision Nacional de Investigacion Cientifica y Tecnologica de Chile.

Disclosures

None.

References

Frequency of Familial Hyperaldosteronism Type 1 in a Hypertensive Pediatric Population: Clinical and Biochemical Presentation

Marlene Aglony, Alejandro Martínez-Aguayo, Cristian A. Carvajal, Carmen Campino, Hernán García, Rodrigo Bancalari, Lillian Bolte, Carolina Avalos, Carolina Loureiro, Pamela Trejo, Karin Brinkmann, Vinka Giadrosich, Verónica Mericq, Ana Rocha, Alejandra Avila, Viviana Pérez, Andrea Inostroza and Carlos E. Fardella

Hypertension. 2011;57:1117-1121; originally published online April 18, 2011;
doi: 10.1161/HYPERTENSIONAHA.110.168740

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/57/6/1117

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2011/04/15/HYPERTENSIONAHA.110.168740.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
ONLINE SUPPLEMENTAL DATA

FREQUENCY OF FAMILIAL HYPERALDOSTERONISM TYPE 1
IN A HYPERTENSIVE PEDIATRIC POPULATION:
CLINICAL AND BIOCHEMICAL PRESENTATION.

Marlene Aglony (1*), Alejandro Martínez-Aguayo (1*), Cristian A. Carvajal (2), Carmen Campino (2), Hernán García (1), Rodrigo Bancalari (1), Lillian Bolte (1), Carolina Avalos (3), Carolina Loureiro (1), Pamela Trejo (2), Karin Brinkmann (4), Vinka Giadrosich (5), Verónica Meriq (6), Ana Rocha (6), Alejandra Avila (6), Viviana Perez (1), Andrea Inostroza (7) and Carlos E. Fardella (2).

*Authors contributed equally to this work

1 Division of Pediatrics, Pontificia Universidad Católica de Chile. Santiago, Chile.
2 Department of Endocrinology, Pontificia Universidad Católica de Chile. Santiago, Chile.
3 Department of Pediatrics, Universidad de Antofagasta, Chile.
4 Department of Pediatrics, Hospital Regional de Punta Arenas. Punta Arenas, Chile.
5 Department of Pediatrics, Universidad de Valparaiso. Valparaíso, Chile.
6 Institute of Maternal and Child Research (IDIMI), Faculty of Medicine, University of Chile. Santiago, Chile.
7 Department of Nephrology, Pontificia Universidad Católica de Chile. Santiago, Chile.

Abbreviated title: Familial hyperaldosteronism type 1

Correspondence author: Carlos Fardella, MD
Endocrinology Department, Faculty of Medicine
Pontificia Universidad Católica de Chile
Lira 85, piso 5, Santiago, CHILE
PHONE: (56-2) 354-3095 FAX: (56-2) 638-5675
E-mail: cfardella@med.puc.cl
EXPANDED PATIENTS AND METHODS

Excluded patients
We excluded 18 patients due to the following reasons: Refused to participate (n=3), severe obesity (n=3), under drugs that might affect the renin-angiotensin-aldosterone system (n=2), incomplete 12 h urinary collection (n=4), high-dose of inhalated corticoids (n=2), William syndrome (n=1), fasting hyperglycemia (n=1), major depressive disorder (n=1) and Polycystic Ovary Syndrome under oral contraceptive treatment (n=1).

Paternity study
In family 3, the parental relationship between the index case and his parents was performed using a paternity study. Genomic DNA was obtained from all of subjects with AmpFISTR®Identifiler®, Applied Biosystems, which includes 15 microsatellite markers (STR) and amelogenin (gender identifier), which resulted in a >99.9% confidence in the parental relationship (1, 2).
EXPANDED RESULTS

Figure S1.- Pedigree from the four families with HF-I.
Females are designated by circles, and males by squares. Affected status is indicated by blackened symbols. A diagonal line through the symbol indicates deceased individuals. The arrow indicates the index case. Patients F3:I-1 and F4:I-3 (shaded symbols) were not tested.
Effects on Atrial Fibrillation in Aged Hypertensive Rats by Ca2+-activated K+ channel inhibition

Running title: Effects on AF in Aged SHR by $I_{KCa}$ inhibition

Jonas Goldin Diness, MSc$^{1,2}$, Lasse Skibsbye, MSc$^1$, Thomas Jespersen, PhD$^2$, Emil Daniel Bartels, PhD$^3$, Ulrik S. Sørensen, PhD$^1$, Rie Schultz Hansen, PhD$^4$, Morten Grunnet, PhD$^{1,2}$

$^1$NeuroSearch A/S, Pederstrupvej 3, 2750 Ballerup, Denmark

$^2$The Danish National Research Foundation Center for Cardiac Arrhythmia, The Panum Institute, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark

$^3$Department of Clinical Biochemistry, University Hospital of Copenhagen, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark

$^4$Zealand Pharma A/S, Smedeland 36, 2600 Glostrup, Denmark

Correspondence: Morten Grunnet, NeuroSearch A/S, Pederstrupvej 93, 2750 Ballerup, Denmark. Phone: +45 4460 8706. Fax: +45 4460 8080

Email: mgr@neurosearch.dk
Expanded Materials and Methods

In vivo experiments were conducted as previously described with a few modifications (1). The rats were mebumal-anaesthetized (50 mg/kg, i.p.), artificially respirated and kept at 37°C. Tail and pod reflexes were tested frequently to assure effective anaesthesia. At the end of each experiment the hearts were excised and weighed. Following thoracotomy, a pacing electrode was placed on the right atrium (RA), monophasic action potential (MAP) recordings were obtained with a MAP electrode placed on the RA and an electrocardiogram (ECG) was obtained with subcutaneous needle electrodes, two near the forelimbs and one near the left hind limb. After a period of stabilization without pacing, the aERP was measured by continuously pacing the RA at a rate of 500 beats per minute (BPM) and applying 10 basic stimuli (S1) followed by premature S2 stimuli applied with 1-10 ms decrements. The aERP was defined as the longest S1-S2 coupling interval failing to elicit an extra action potential. The Wenckebach cycle length (WCL) was measured by pacing the RA with increasing frequencies, and the WCL was defined as the slowest pacing rate failing to conduct 1:1 to the ventricles.

Having determined the aERP and WCL, continuous pacing was stopped and short episodes of AF were induced every 2 minutes by open-chest burst pacing (83 Hz) of the RA for 10 s followed by 110 s of intrinsic heart rhythm. Animals were divided into three groups receiving i.v. injections of either NS8593 (5 mg/kg), UCL1684 (3 mg/kg), or vehicle subsequent to 30 minutes of baseline recordings. The total duration of AF at all 110 s interburst intervals was measured over a period 30 minutes before injection in order to establish the baseline AF duration. After injection, aERP and WCL were measured as well as the total duration of AF over another period of 30 minutes.
Supporting information

Blood pressure, weight, and hypertrophy

Background data such as sBP, weight, heart weight, and SR can be seen in Table S1. As expected, the recordings of sBP revealed consistently higher values in the SHRs compared to the WKYs at all ages (p<0.001 for all ages of SHR compared with all ages of WKY). In the SHR the sBP increased between ages 3 and 8 months (192±9mmHg and 212±12mmHg, respectively,), whereas the sBP decreased in the WKY between ages 3 and 8 months (134.9±2.6mmHg and 120.7±3.0mmHg). No age-related changes in sBP were observed between 8 and 11 months of age within the two strains.

The WKYs generally weighed more than the age-matched SHRs and they gained weight at a more rapid rate than the SHRs. Two-way ANOVA reveals that strain, age, and interaction accounts for 21.1%, 62.0%, and 3.7%, respectively, of the total variance seen in the weight measurements (p<0.0001 in all cases). The Bonferroni post hoc test showed statistically significant differences between the strains at the age of 8 and 11 months with p-values below 0.001, whereas the strain-related weight difference at 3 months was not statistically significant.

The heart weight:body weight ratios demonstrated that hearts from SHRs were hypertrophied in comparison with WKY rats which is consistent with previous reports(2-4). Recordings of SR from conscious and anaesthetized animals differed somewhat; in the conscious animals the recordings showed slightly faster heart rates in the SHR as compared to age-matched WKY. Only one statistically significant difference in SR was observed in the awake animals, namely a significantly faster heart rate in the SHR8 than in the WKY8.

The baseline recordings of SR from anaesthetized animals revealed faster heart rates in the SHR3 as compared to WKY3, no difference in SR between SHR8 and WKY8, and slower hearts rates in SHR11 than WKY11. The SHR11 had significantly slower heart rates at the baseline recordings than both the WKY11 and the younger groups of SHR. Previous recordings from isolated atrial preparations from WKY and SHR aged 5-6 months show no differences in basal heart rates. However, a hyperresponsiveness to norepinephrine-induced increase in SR was observed in SHR as compared to WKY, which might explain these differences in heart rate between anaesthetized and awake animals(5).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 months</th>
<th></th>
<th>8 months</th>
<th></th>
<th>11 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>134.9</td>
<td>2.6</td>
<td>120.7</td>
<td>3.0†</td>
<td>120.0</td>
<td>3.1†</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>291.4</td>
<td>3.9</td>
<td>479.4</td>
<td>13.7</td>
<td>541.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.49</td>
<td>0.06</td>
<td>1.51</td>
<td>0.06</td>
<td>1.74</td>
<td>0.07†‡</td>
</tr>
<tr>
<td>Heart weight/body weight (g/kg)</td>
<td>4.99</td>
<td>0.21</td>
<td>3.16</td>
<td>0.10†</td>
<td>3.21</td>
<td>0.09†‡</td>
</tr>
<tr>
<td>SR awake (BPM)</td>
<td>379.7</td>
<td>8.12</td>
<td>372.2</td>
<td>7.01</td>
<td>372.6</td>
<td>6.77</td>
</tr>
<tr>
<td>SR baseline (BPM)</td>
<td>326.1</td>
<td>11.4</td>
<td>364.1</td>
<td>8.9</td>
<td>359.4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Table S1
Background data for the different strain and age groups.
<table>
<thead>
<tr>
<th>Strain, age, and treatment</th>
<th>Baseline aERP (ms)</th>
<th>Treatment aERP (ms)</th>
<th>Baseline AF (s)</th>
<th>Treatment AF (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR3 NS8593</td>
<td>30.2 ± 9.8</td>
<td>60.3 ± 9.6</td>
<td>2.87 ± 2.01</td>
<td>0.36 ± 0.46</td>
</tr>
<tr>
<td>SHR3 UCL1684</td>
<td>28.6 ± 4.5</td>
<td>57.0 ± 28.7</td>
<td>2.44 ± 0.27</td>
<td>0.61 ± 0.52</td>
</tr>
<tr>
<td>SHR3 Vehicle</td>
<td>31.8 ± 8.5</td>
<td>33.7 ± 1.5</td>
<td>1.47 ± 0.60</td>
<td>1.59 ± 1.16</td>
</tr>
<tr>
<td>SHR8 NS8593</td>
<td>33.3 ± 12.6</td>
<td>57.0 ± 23.6</td>
<td>3.11 ± 0.67</td>
<td>0.63 ± 0.45</td>
</tr>
<tr>
<td>SHR8 UCL1684</td>
<td>25.3 ± 6.1</td>
<td>47.0 ± 18.4</td>
<td>4.03 ± 0.76</td>
<td>1.15 ± 0.86</td>
</tr>
<tr>
<td>SHR8 Vehicle</td>
<td>23.4 ± 5.5</td>
<td>29.0 ± 9.6</td>
<td>2.51 ± 0.65</td>
<td>2.85 ± 0.84</td>
</tr>
<tr>
<td>SHR11 NS8593</td>
<td>21.5 ± 4.2</td>
<td>44.0 ± 6.7</td>
<td>2.80 ± 0.70</td>
<td>0.71 ± 0.93</td>
</tr>
<tr>
<td>SHR11 UCL1684</td>
<td>27.0 ± 8.9</td>
<td>54.5 ± 15.4</td>
<td>7.22 ± 7.61</td>
<td>0.58 ± 0.46</td>
</tr>
<tr>
<td>SHR11 Vehicle</td>
<td>24.8 ± 5.3</td>
<td>28.5 ± 6.2</td>
<td>2.71 ± 0.74</td>
<td>2.88 ± 0.62</td>
</tr>
<tr>
<td>WKY3 NS8593</td>
<td>45.8 ± 13.5</td>
<td>74.0 ± 13.3</td>
<td>0.59 ± 0.62</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>WKY3 UCL1684</td>
<td>36.0 ± 5.6</td>
<td>55.8 ± 3.0</td>
<td>1.71 ± 0.81</td>
<td>0.62 ± 0.40</td>
</tr>
<tr>
<td>WKY3 Vehicle</td>
<td>38.0 ± 18.4</td>
<td>49.3 ± 14.0</td>
<td>1.07 ± 1.00</td>
<td>0.91 ± 0.96</td>
</tr>
<tr>
<td>WKY8 NS8593</td>
<td>27.2 ± 10.7</td>
<td>40.0 ± 15.6</td>
<td>3.29 ± 0.63</td>
<td>1.31 ± 0.50</td>
</tr>
<tr>
<td>WKY8 UCL1684</td>
<td>33.6 ± 8.9</td>
<td>46.8 ± 13.8</td>
<td>4.93 ± 4.10</td>
<td>1.39 ± 0.90</td>
</tr>
<tr>
<td>WKY8 Vehicle</td>
<td>32.0 ± 7.0</td>
<td>40.2 ± 9.9</td>
<td>2.40 ± 0.64</td>
<td>3.00 ± 0.68</td>
</tr>
<tr>
<td>WKY11 NS8593</td>
<td>34.2 ± 3.8</td>
<td>38.3 ± 13.1</td>
<td>2.48 ± 0.70</td>
<td>0.54 ± 0.38</td>
</tr>
<tr>
<td>WKY11 UCL1684</td>
<td>34.2 ± 6.8</td>
<td>40.4 ± 6.0</td>
<td>2.63 ± 0.48</td>
<td>0.30 ± 0.20</td>
</tr>
<tr>
<td>WKY11 Vehicle</td>
<td>35.3 ± 8.6</td>
<td>36.8 ± 12.6</td>
<td>1.65 ± 0.63</td>
<td>1.82 ± 0.66</td>
</tr>
</tbody>
</table>

**Table S2**
AF durations and aERP values presented individually for each animal strain, age group and treatment group.
Reference List


