Polymorphisms in the *RAC1* Gene Are Associated With Hypertension Risk Factors in a Chilean Pediatric Population

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BACKGROUND
The GTPase Rac1 has been implicated in hypertension as a modulator of mineralocorticoid receptor activity. Our aim is to investigate the frequency of polymorphisms rs10951982 (intron 1, G>A) and rs836478 (intron 3, T>C) in the *RAC1* gene and perform association studies with clinical and biochemical parameters in a Chilean pediatric cohort.

METHODS
Two hundred two normotensive (NT) subjects (aged 4–16 years) were divided into 2 groups: NT subjects with hypertensive parents (NH; n = 103) and NT subjects with NT parents (NN; n = 99). We measured markers of inflammation (high-sensitivity C-reactive protein, interleukin 6 (IL-6), interleukin 8, and tumor necrosis factor α), endothelial damage (Plasminogen activator inhibitor-1 metalloproteinase-9, and metalloproteinase-2), and oxidative stress (malondialdehyde). Data were expressed as median and interquartile range (IQR).

RESULTS
We found differences in polymorphism rs836478 (intron 3, C>T) in both genotypic (χ^2 = 15.2, 2 df; P = 0.0005) and allelic (X^2=5.5, 1 df; P = 0.01) frequencies in NH vs. NN subjects. NH subjects with a TT genotype showed increase MMP9 expression (median = 2.3, IQR - 1.6–3.2; vs. median = 1.6, IQR = 1.6–2.3 AU; P = 0.01) and lower IL-6 expression (median = 8.8, IQR = 7.0–11.8; vs. median = 12.1, IQR = 8.2–14.7 pg/ml; P = 0.02) compared with subjects with TC/CC genotype. No difference in the allelic frequency distribution was seen in the polymorphism rs10951982 (NH vs. NN: χ_2=0.2, 1 df; P = 0.6). For this SNP, NN subjects with GA/AA genotype showed decreased diastolic BP indexes compared with subjects with native GG genotype (median = 1.08, IQR = 1.0–1.2; vs. median = 0.99, IQR = 0.94–1.1; P = 0.02).

CONCLUSIONS
We report the frequency of polymorphisms rs836478 and rs10951982 of the *RAC1* gene in a Spanish-Amerindian cohort. The polymorphism rs836478 was associated with an increased expression in markers of inflammation and endothelial damage (MMP9 and IL-6) in pediatric subjects with a hypertensive genetic background.

Keywords: blood pressure; hypertension; normotensive; PCR-HRM; polymorphisms; *RAC1* gene.

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The prevalence of hypertension in children has increased in the world over the last 20 years, most likely because of the dramatic increase in childhood obesity.[1] However, genetic and environmental factors may also explain the increased prevalence of hypertension in children.[2,3]

Hypertension is a known risk factor for cardiovascular disease (CVD) in adults, and the presence of childhood hypertension may contribute to the early development of CVD. Patients with severe cases of childhood hypertension are also at increased risk of developing hypertensive encephalopathy, seizures, cerebrovascular accidents, and congestive heart failure. Based on these observations, early detection and treatment in children with hypertension should improve the long-term complications of hypertension.

Fifteen years ago it was estimated that the prevalence of hypertension in children and adolescents was 1%. However, recent research indicates that the prevalence has risen dangerously worldwide. In 2011, our group identified a hypertension prevalence of 13.6% in a cohort of Chilean pediatric subjects.[4] Considerable efforts have been made in recent years to identify genetic components that contribute to the development of hypertension. Among various mechanisms involved in

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blood pressure regulation, mineralocorticoids have a crucial role in maintaining the homeostasis of salt, water, and vascular tone through the renin–angiotensin–aldosterone system. Aldosterone activates the mineralocorticoid receptor (MR) in epithelial tissues and regulates the sodium transport. The aldosterone/MR complex could also contribute to an inflammatory response, which is characterized by vascular infiltration of immune cells, oxidative stress, and proinflammatory cytokine production and contributes to the damage in renal, heart, and vascular tissue. Although several research groups have proposed that MR biological activity is influenced by factors other than aldosterone, recent studies have revealed a cross-talk between Rac1, a small GTP-binding protein, and MR activation independent of aldosterone, which would be associated with the development of salt-sensitive hypertension and cardiovascular damage.[5]

Rac1 is a member of the Rho family of GTPases, and it cycles between an inactive GDP-bound and an active GTP-bound state.[6] The primary Rac1 transcript gives rise to 2 messenger RNAs: Rac1a (1.2 kD in size) and Rac1b (2.5 kD in size).[7] Rac1a is stabilized in its GDP-bound form in the cytoplasm through an interaction with Rho-GDI.[8] The Rac1b isoform is a variant product that contains an additional 57 nucleotides, resulting in an in-frame insertion of 19 new amino acids.[9] Unlike Rac1a, the Rac1b isoform displays a faster GDP/GTP exchange rate, does not bind Rho-GDI, and does not require external stimulus for activation, and is therefore constitutively bound to the plasma membrane.[6,10]

Rac1 is part of the NADPH oxidase complex, which induces the generation of reactive oxygen species, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which lead to increased oxidative stress, alterations in the cell membrane, and endothelial damage. In fact, oxidative stress has been shown to play a key role in cardiac pathologies associated with MR,[11] possibly through the activation of nuclear factor B (NF-kB), an important transcription factor of many proinflammatory cytokines.[12] However, few reports investigate the regulation of Rac1 expression through an MR-dependent mechanism in renal disease.

The importance of inflammation and oxidative stress in human cardiovascular disease is supported by studies from Zalba *et al.*,[13] who demonstrated that polymorphisms in NADPH oxidase subunits are associated with increased atherosclerosis and hypertension. However, hypertension has not been associated with polymorphisms in the *RAC1* gene, but as observed previously, it has been proposed that alterations of Rac1, by impairment of its activity, in its expression, or of its associated proteins (e.g., GEFs), could influence susceptibility to diseases such as renal and/or cardiac failure or hypertension.[14] Rac1 is implicated in a variety of diseases, and it is known that single nucleotide polymorphisms (SNPs) may confer disease susceptibility, as well as determine the disease severity and progression. In 2011, a study genotyping the polymorphism rs10951982 (G>A) located in intron 1 of *RAC1* was conducted in a European cohort, which demonstrated a decrease in *RAC1* gene expression in peripheral leukocytes.[15]

In this study, we evaluated 2 polymorphisms in the *RAC1* gene (NG_029431) that could affect the Rac1 protein expression and then determined the association of both polymorphisms with hypertension, endothelial damage, and inflammation in a Chilean pediatric population.

METHODS

Subjects

This study was designed as a cross-sectional study. Chilean children and adolescents of both sexes, ranging in age from 4 to 16 years, were invited to participate. We recruited 202 normotensive pediatric subjects with lower-middle, middle, and high socioeconomic status.

Trained nurses measured the blood pressure (BP) and heart rate in all subjects. Three measurements were obtained from the right arm at consecutive 5-minute intervals using an oscillometric method (Dinamap CARESCAPE V100; GE Healthcare, Medical Systems Information Technologies, Milwaukee, WI) with the subjects in a seated position. This measurement was performed with a cuff and bladder that were size-adjusted to the subject's upper-arm girth according to the published recommendations.[16,17] Normotension in children was defined as an average systolic BP (SBP) and/or diastolic BP (DBP) <90th percentile for sex, age, and height. The BP index was determined using the observed BP/50th percentile BP level for sex, age, and stature using the normal values reported.[16] In adults, normotension was considered to be at least 2 different measurements of BP <140/90 mm Hg. Two subgroups of subjects were defined according to the BP of their parents: normotensive children with hypertensive parents (NH; n = 103) and normotensive children with normotensive parents (NN; n = 99). Clinical characteristics of subjects involved in the study are described in Table 1.

Normotensive subjects in this study have an ethnic origin of Spanish/white-Amerindian. Briefly, the Chilean population originated through a biracial mixture of genes originating from the Spanish conquerors and a gene pool derived from the native Amerindians.[18,19]

The protocol followed in this study was written according to the guidelines of the Declaration of Helsinki and

Table 1. Clinical characteristics of children stratified by hypertension family history

Parameters	NH	NN
No.	103	99
Female sex, %	55.3	58.3
Age, y	11.5 (9.2 to13.1)	10.65 (8.55 to 12.8)
Height, SDs	0.12 (−0.45 to 0.81)	0.35 (−0.32 to 0.83)
BMI, percentile	81.0 (49.3 to 94.3)	80.25 (53.51 to 94.19)
SBP, index	1.04 (1.0 to 1.8)	1.03 (0.96 to 1.07)
DBP, index	1.08 (1.01 to 1.15)	1.05 (0.98 to 1.14)

Values correspond to median (interquartile range). Mann–Whitney test.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; NH, normotensive children with hypertensive parents; NN, normotensive children with normotensive parents.

approved by the Ethical Committee of the Faculty of Medicine, Pontificia Universidad Catolica de Chile. The study and protocol were explained to all participants, and written informed consent was obtained.

Biochemical and hormonal analysis

A total of 202 healthy, normotensive children were enrolled in the study. After an overnight fast, basal blood samples were obtained between 8:00 and 10:00 AM. Serum aldosterone was assayed using a commercial radioimmunoassay kit (Coat-A-Count Kit; Siemens, Los Angeles, CA). Based on our previous reports in normotensive children, plasma renin activity was measured by radioimmunoassay using a commercial kit (DiaSorin, Stillwater, MN).[20] The lower limit of the plasma renin activity assay was 0.1 ng/ml/hour.[21] Interleukin 6 (IL-6) was measured with an enzyme-linked immunosorbent assay (ELISA) using commercial reagents and standards according to the manufacturer's protocols (Bender Med Systems, Vienna, Austria). Interleukin 8 and tumor necrosis factor α (TNF-α) were measured with an ELISA using commercial kits (eBioscience, Vienna, Austria). Plasminogen activator inhibitor-1 (PAI-1) was measured with an ELISA (HYPHEN BioMed, Neuville sur Oise, France). Metalloproteinase-9 (MMP9) and metalloproteinase-2 (MMP2) activities were estimated by zymography, as described previously.[22] The results are expressed as arbitrary units of the number of changes with respect to the reference plasma used as an internal control. High-sensitivity C-reactive protein (hs-CRP) was measured with a nephelometric assay (BN ProSpec Systems; Siemens Healthcare Diagnostics Products, Marburg, Germany). The malondialdehyde (MDA) measurement quantifies the thiobarbituric acid-reactive compounds, as previously described.[23]

At the same time, 12-hour nocturnal urine (between 7:00 PM and 7:00 AM) samples were collected. Total 12-hour urine volumes were measured, and 50-ml aliquots were stored to measure creatinine and sodium. Urinary creatinine was measured by the Jaffe method with automated equipment (Modular Analytics, Roche, Germany). We also calculated the fractional sodium excretion. The serum, plasma, and urine samples were stored at −70°C until analysis.

Genetic analysis

Genomic DNA was isolated from peripheral leucocytes with the Lahiri method.[24] Real-time polymerase chain reaction (PCR) amplification of the intronic region (intron 1 and 3) of the *RAC1* gene was performed using the RotorGene 6000 instrument (Corbett Research, Sydney, Australia). Hybridizing primers were designed to amplify both *RAC1* gene polymorphisms: rs10951982, located in intron 1, and rs836478, located in intron 3. The primer sequences used were as follows: intron 1 (forward: 5'-CAGCAGGCAGAGGTTGTGGTA-3'; reverse: 5'-CATGAGTCGGCTGGAAGAAAGTTC-3′; size: 258bp) and exon-intron 3 boundary region (forward: 5′-CGGT GAATCTGGGCTTATG-3'; reverse: 5′- GCAAGTCCATAG GTAACATG-3′; size: 163bp). The reaction mixture contained 12.5 µl of sensimix High Resolution Melting (HRM) 2×, 1 µl of EvaGreen fluorescent dye (both reagents as part of the SensiMix HRM Kit; Bioline USA, Tauton, MA), 0.5 µM of both forward and reverse primers, 0.5 µl Gotaq (GoTaq Colorless Master Mix; Promega, Madison, WI), 2 µl of DNA (100 ng/µl), and nuclease-free water up to 25 µl of the final volume. Three positive and negative controls were included (samples of DNA with each of the known genotypes) in each run. Thermal cycling consisted of an initial 8-minute hold at 95 °C, followed by a 15-second hold at 95 °C, a 15-second hold at 56 °C, and a 20-second hold at 72 °C for 40 cycles. Finally, the HRM step consisted of a ramp from 70 °C to 95 °C, rising 0.15 seconds at each step. Samples were analyzed with ClustalW software, and sequences were confirmed by Sanger sequencing at Macrogen (Seoul, Korea).

Data analysis

Differences in genotype frequencies in *RAC1* polymorphisms were analyzed by χ^2. A Hardy–Weinberg analysis was performed to identify the correspondence between the observed and calculated genotypes. The odds ratio (OR) identified the probability of the presence of the less prevalent allele, and a 95% confidence interval (CI) was used to estimate the precision of the OR. The results were expressed as median values (interquartile range (Q1–Q3)). The comparison between subject groups was performed using the Kruskal–Wallis and Dunn's multiple comparisons tests with GraphPad Prism version 5.0 software (San Diego, CA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Identification and genotyping of *RAC1* polymorphisms in pediatric subjects

We successfully identified both rs10951982 and rs836478 SNPs in the *RAC1* gene in Chilean normotensive subjects using PCR-HRM technology. The PCR products, which were further analyzed by HRM, displayed 3 different melting curves that represented the 3 possible genotypes (homozygous, heterozygous, and alternative homozygous): GG, GA, and AA for the SNP rs10951982 and TT, TC, and CC for the SNP rs836478, respectively (Figure 1).

Frequency and genotype distribution

SNP rs10951982 in intron 1. The genotype distribution (GG, GA, AA) of the SNP rs10951982 (intron 1) was similar among subjects in the NH (68%, 30%, 2%, respectively) and NN groups (64%, 31%, 5%, respectively), and the allelic frequency distribution was also similar between both groups. We showed that the 2 groups of subjects were in Hardy–Weinberg equilibrium. The minor allele frequency, in this case that of adenine (A), was 0.17 and 0.20 for the NH and NN groups, respectively. These data were then analyzed by the OR method, which did not show any differences between the groups (Table 2).

SNP rs836478 in intron 3. The genotype distribution (TT, TC, CC) of the SNP rs836478 (intron 3) was different among subjects in the NH (25%, 45%, 29%, respectively) and NN groups (45%, 44%, 10%, respectively; $P = 0.0005$)

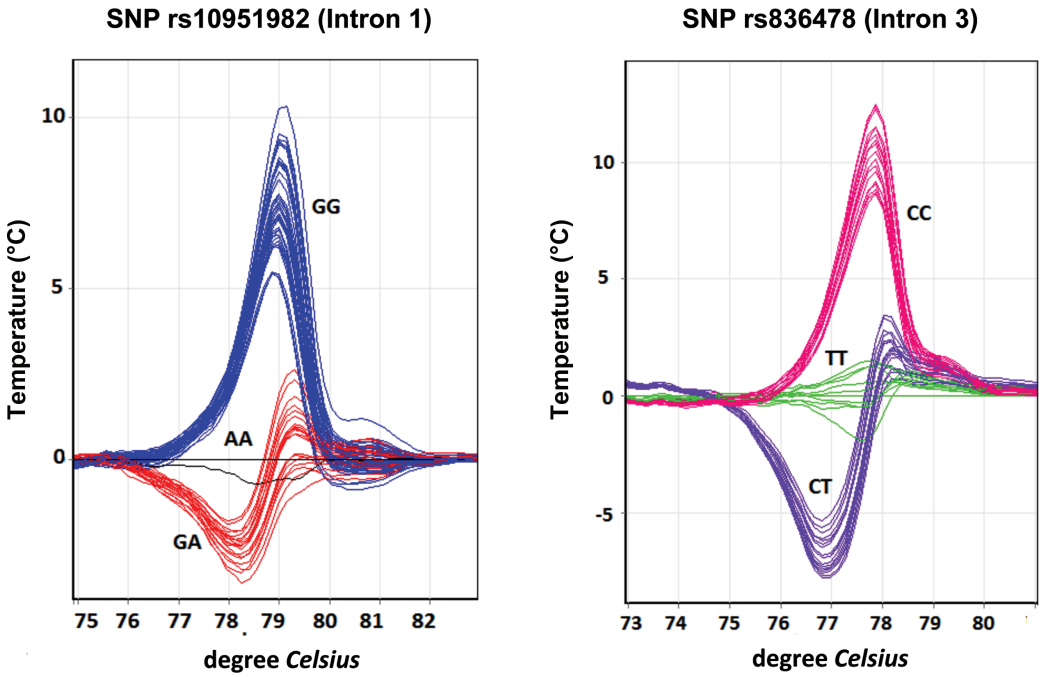

Figure 1. Temperature curves by High Resolution Melting (HRM) of 2 polymorphisms of *RAC1* gene. DNA was analyzed in 202 pediatric subjects by polymerase chain reaction–HRM. (**a**) Single nucleotide polymorphism (SNP) rs10951982 (intron 1, *RAC1*). Blue: native genotype (GG). Red: heterozygous genotype (GA). Coffee: alternative genotype (AA). (**b**) SNP rs836478 (intron 3, *RAC1*). Pink: native genotype TT. Purple: heterozygous genotype TC. Green: CC alternative genotype.

(Table 2). We showed that the 2 groups of subjects were in Hardy–Weinberg equilibrium. The minor allele frequency, in this case that of cytosine (C), was 0.52 in the NH group and 0.32 in the NN group (Table 2). These data were also analyzed by the OR method, and the differences between groups were shown. The C allele occurred less frequently in the NN group than in the NH group (OR = 0.576; 95% CI = 0.36–0.92).

Associations of clinical and biochemical parameters

Biochemical parameters were analyzed in 202 normotensive children. We analyzed the association of polymorphisms with different clinical and biochemical parameters and also with oxidative stress, inflammation, endothelial dysfunction, and myocardial remodeling markers. The results are shown in Tables 3 and 4. For the polymorphism rs10951982 (intron 1), we found that NN subjects with GA/AA genotypes had a lower diastolic blood pressure index compared with NN subjects with the GG native genotype (median = 1.0, IQR = 0.95–1.10; vs. median = 1.075, IQR =1.01–1.16; $P = 0.03$) (Figure 2). However, we did not find any significant differences in parameters tested in the NH group (Table 3).

For the polymorphism rs836478 (intron 3), we found that NN subjects with the alternative genotype (TT) had increased PAI-1 expression levels compared with NN subjects with the TC/CC genotype (median = 16.39, IQR =9.61–26.64; vs. median = 12.49, IQR = 7.96–18.88 ng/ml; $P = 0.04$) (Figure 2). In the NH group, subjects with the alternative genotype (TT) had decreased IL-6 expression levels compared with NH subjects with the TC/CC genotype (median = 8.8, IQR = 7.0–11.8; vs. median = 12.1, IQR = 8.3–14.6 pg/ml; $P = 0.007$) (Figure 2). MMP9 activity was increased in NH subjects with the TT genotype compared with NH subjects with the TC/CC genotype (median = 2.3, IQR = 1.6–3.2; vs. median = 1.6, IQR = 1.2–2.4 AU; $P = 0.004$) (Figure 2).

DISCUSSION

We studied and genotyped 2 polymorphisms in the *RAC1* gene in a Chilean pediatric cohort. The SNP rs10951982 located in intron 1 had similar allelic and genotypic frequency between groups. However, we observed differences in the allelic frequency and genotypic distribution for the SNP rs836478, located in intron 3 of the *RAC1* gene, between the NH and NN subjects. Additionally, both polymorphisms had a minor allele frequency similar to that which was published in the databases SNPs NCBI (www.ncbi.nlm.nih.gov/projects/SNP), HAPMAP (www.hapmap.ncbi.nlm.nih.gov/), and ENSEMBL (www.ensembl.org) for the Tuscan, European, and American populations.

We found an association between blood pressure and the *RAC1* intron 1 polymorphism (rs10951982) in NN children. The DBP index was decreased in NN subjects with the GA/AA genotype compared with subjects with the native GG genotype, suggesting the alternative A allele could be associated with normotension. One study supports this observation to date, demonstrating the SNP rs10951982 and its association with the expression of *RAC1* decreases in the presence of the AA genotype.[15] In the pediatric cohort studied here, the presence of the polymorphism rs10951982

Table 2. Genotype distribution and allelic frequencies of the polymorphisms of *RAC1* gene in Chilean pediatric cohort

SNP	Location	Genotype frequencies	NH	NN	χ^2, df	*P* value	Allelic frequencies	NH	NN	MAF	OR (95% CI)	χ^2, df	*P* value
rs10951982	Intron 1 (G/A)	GG	70 (68%)	54 (64%)	1.25, 2	0.53	G	101 (75.4%)	80 (72.7%)	0.18	1.15 (0.65–2.04)	0.22, 1	0.64
		GA	31 (30%)	26 (31%)									
		AA	2 (2%)	4 (5%)			A	33 (24.6%)	30 (27%.3)				
		GA + AA	33 (32%)	30 (36%)									
rs836478	Intron 3 (T>C)	TT	27 (25%)	45 (45.5%)	15.21, 2	0.0005	T	75 (48.7%)	89 (62.2%)	0.42	0.578 (0.36–0.92)	5.49, 1	0.02
		TC	48 (45%)	44 (44.5%)									
		CC	31 (29%)	10 (10.1%)			C	79 (51.3%)	54 (37.8%)				
		TC + CC	79 (75%)	54 (55%)									

Abbreviations: MAF, minor allelic frequency; NH, normotensive with hypertensive parents; NN, normotensive with normotensive parents; df, degree of freedom.

(G>A) lowered the DBP and might confer protection from hypertension in the vasculature of subjects with this polymorphism. Molecular explanations for this finding are speculative. The results from the bioinformatic assays show the SNP rs10951982 is located in a cis regulatory site in intron 1 of *RAC1* gene and may also decrease the *RAC1* gene expression in the presence of this polymorphism (data not shown).[15,25] Although the polymorphism rs10951982 (GA/AA) is associated with low BP in NN subjects, this SNP by itself cannot fully explain the association with BP and may require additional protector factors, which should be inherited from the subjects' normotensive parents.

The SNP rs836478 located in intron 3 was associated with some markers of inflammation and endothelial damage, which suggests the effects of Rac1 could be primarily at the vascular level. Indeed, some studies have shown that Rac1 has a pivotal role in the regulation of the gene expression involved in vascular cell function and inflammation,[26,27] which would be associated with the hypertensive disease.[28] We found that NN subjects carrying the TT genotype have increased PAI-1 expression levels compared with NN subjects with the TC/CC genotype. Interestingly, previous studies have shown that Rac1-dependent reactive oxygen species formation promotes PAI-1 expression and increases its promoter activity in both endothelial and smooth muscle cells.[29–31] Thus, subjects carrying the TT genotype (SNP rs836478) would have increased PAI-1 expression levels, which is associated with a major risk for thrombosis and endothelial dysfunction.

We found that NH subjects carrying the rs836478 polymorphism (TT genotype) have decreased IL-6 levels compared with NH subjects with the TC/CC genotype, which may lead to increased inflammatory activity. IL-6 has significant anti-inflammatory effects, including the ability to induce synthesis of the interleukin 1 receptor antagonist to induce the release of soluble TNF receptors[32] and to inhibit TNF production of proinflammatory cytokines and MIP2.[33,34] Therefore, decreasing IL-6 would compromise the development of hypertension because inflammatory mechanisms affect certain concentrations of cytokines that are crucial in the development and progression of organ damage in hypertensive patients.[35]

This study also showed an elevation of MMP9 expression in NH subjects carrying a TT homozygous genotype compared with NH subjects with a TC/CC genotype, which could be an important risk factor in the development of hypertension because MMP9 is involved in extracellular matrix degradation, as well as in tissue remodeling, with both phenomena contributing to organ dysfunction (e.g., heart) and increasing the fibrosis.[36] These results are supported by previous studies showing that MMP9 levels are higher in hypertensive subjects than in normotensive control subjects.[37,38] MMP9 is released from endothelial and smooth muscle cells after stimulation by hypertension-related mechanical stress on the vascular wall. In normotensives subjects, our data make it possible to suggest that the presence of the SNP rs836478 TT genotype could increase the expression of *RAC1* and the production of reactive oxygen species through NADPH oxidase signaling and NFκB activation, which regulates MMP9 activity, providing a link between oxidative stress and

Table 3. Clinical and biochemical characteristics of normotensive subjects classified according to their genotypes for SNP rs10951982 located in intron 1 of the *RAC1* gene

	NH			NN		
Parameter	**GG**	**GA + AA**	***P* value**	**GG**	**GA + AA**	***P* value**
SBP index	1.04 (1.00–1.08)	1.04 (0.96–1.09)	0.40	1.02 (0.96–1.07)	1.03 (0.97–1.06)	0.90
DBP index	1.07 (1.00–1.15)	1.08 (1.00–1.14)	0.86	1.07 (1.01–1.16)	1.00 (0.96–1.10)	0.02
Serum aldosterone, ng/dl	6.30 (3.50–9.35)	5.70 (3.05–8.05)	0.44	6.55 (3.17–9.42)	5.25 (3.02–8.52)	0.66
PRA, ng/ml/hour	2.30 (1.60–3.02)	2.30 (1.40–3.10)	0.95	2.45 (1.87–4.12)	2.30 (1.37–2.82)	0.14
hsCRP, mg/L	0.34 (0.22–1.20)	1.12 (0.20–2.31)	0.13	0.41 (0.22–1.22)	0.61 (0.20–1.40)	0.81
MMP9 activity	1.53 (1.09–2.46)	2.02 (1.55–2.57)	0.15	1.76 (1.15–2.43)	1.72 (1.23–2.28)	0.62
MMP2 activity	1.63 (1.10–2.06)	1.54 (1.22–1.79)	0.56	1.53 (1.08–1.83)	1.42 (1.10–1.75)	0.47
PAI-1, ng/ml	19.74 (9.93–30.71)	18.77 (9.91–24.41)	0.50	14.31 (8.16–22.53)	14.34 (8.99–25.54)	0.86
TNF-α, pg/ml	17.31 (11.55–21.44)	17.32 (10.46–24.54)	0.83	16.91 (11.28–21.38)	16.91 (12.03–23.79)	0.93
IL-6, pg/ml	11.57 (7.66–14.51)	11.29 (7.90–14.84)	0.76	11.46 (8.36–16.50)	12.00 (9.28–14.85)	0.84
IL-8, pg/ml	18.20 (13.93–24.91)	21.57 (14.27–32.16)	0.11	19.59 (15.43–26.07)	18.37 (16.32–29.42)	0.55
MDA, μM	0.37 (0.28–0.43)	0.35 (0.26–0.52)	0.52	0.39 (0.27–0.59)	0.35 (0.28–0.48)	0.34
Sodium excretion, mEq/g creatinine	176.00 (129.90–265.50)	193.50 (117.10–254.40)	0.98	182.80 (128.70–240.70)	143.80 (116.10–248.10)	0.37
FeNa 12 h, %	0.64 (0.47–0.87)	0.65 (0.45–0.85)	0.88	0.68 (0.50–0.86)	0.55 (0.41–0.86)	0.24

Values correspond to median (interquartile range).

Abbreviations: DBP, diastolic blood pressure; FeNa, fractional excretion of sodium; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IL-8, interleukin 8; MDA, malondialdehyde; MMP2, metalloproteinase-2; MMP9, metalloproteinase-9; NH, normotensive children with hypertensive parents; NN, normotensive children with normotensive parents; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; SBP, systolic blood pressure; TNF-α, tumor necrosis factor α.

Table 4. Clinical and biochemical characteristics of normotensive subjects classified according to their genotypes for SNP rs836478 located in intron 3 of the *RAC1* gene

	NH			NN		
Parameter	**TT**	**TC + CC**	***P* value**	**TT**	**TC + CC**	***P* value**
SBP index	1.03 (0.99–1.08)	1.05 (1.00–1.09)	0.61	1.00 (0.97–1.08)	1.03 (0.96–1.07)	0.72
DBP index	1.08 (1.01–1.14)	1.08 (0.99–1.15)	0.79	1.03 (0.97–1.15)	1.07 (0.99–1.13)	0.51
Serum aldosterone, ng/dl	6.30 (2.60–8.50)	6.30 (3.50–9.70)	0.69	6.80 (2.95–10.45)	6.60 (4.20–9.50)	0.86
PRA, ng/ml/hr	2.35 (1.62–3.67)	2.30 (1.60–3.10)	0.35	2.40 (1.70–3.05)	2.50 (1.60–4.30)	0.34
hsCRP, mg/L	0.53 (0.22–1.53)	0.52 (0.2–1.87)	0.95	0.66 (0.24–1.65)	0.36 (0.20–1.19)	0.12
MMP9 activity	2.30 (1.59–3.25)	1.58 (1.16–2.37)	0.003	1.83 (1.26–2.50)	1.59 (1.12–2.28)	0.72
MMP2 activity	1.72 (1.15–2.45)	1.47 (1.10–1.87)	0.11	1.50 (1.09–1.79)	1.41 (1.07–1.72)	0.66
PAI-1, ng/ml	19.93 (11.16–30.73)	18.88 (8.56–27.53)	0.39	16.39 (9.61–26.64)	12.49 (7.96–18.88)	0.04
TNF-α, pg/ml	14.67 (8.87–23.11)	17.63 (11.56–22.24)	0.19	15.54 (12.41–22.96)	17.29 (12.03–23.32)	1.00
IL-6, pg/ml	8.83 (7.04–11.84)	12.05 (8.27–14.61)	0.007	12.85 (9.43–16.38)	12.09 (8.71–16.61)	0.91
IL-8, pg/ml	18.95 (14.09–28.83)	16.62 (13.35–24.48)	0.23	20.69 (17.32–28.37)	18.38 (15.02–27.04)	0.11
MDA, μM	0.40 (0.31–0.44)	0.35 (0.26–0.45)	0.40	0.37 (0.27–0.55)	0.38 (0.26–0.54)	0.95
Sodium Excretion, mEq/g creatinine	187.00 (148.10–266.40)	163.80 (120.80–230.90)	0.12	152.10 (111.70–219.50)	154.80 (125.50–227.20)	0.62
FeNa 12h, %	0.72 (0.50–0.91)	0.59 (0.47–0.86)	0.38	0.63 (0.41–0.83)	0.64 (0.47–0.86)	0.84

Values correspond to median (interquartile range).

Abbreviations: DBP, diastolic blood pressure; FeNa, fractional excretion of sodium; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IL-8, interleukin 8; MDA, malondialdehyde; MMP2, metalloproteinase-2; MMP9, metalloproteinase-9; NH, normotensive children with hypertensive parents; NN, normotensive children with normotensive parents; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; SBP, systolic blood pressure; TNF-α, tumor necrosis factor α.

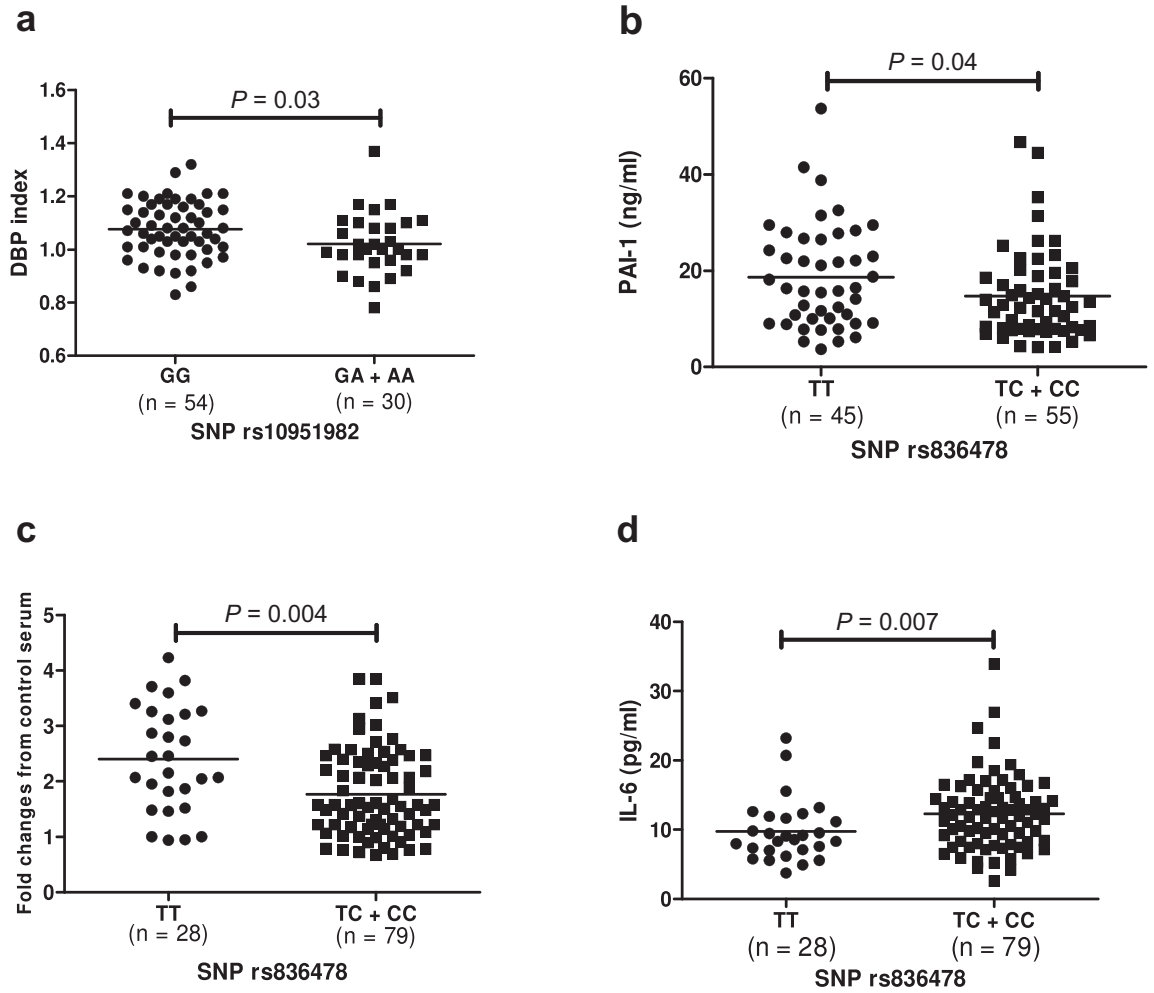

Figure 2. Genotype distribution and biochemical associations of the polymorphisms rs10951982 and rs836478 of the *RAC1* gene in normotensive subjects with hypertensive parents (NH) and normotensive subjects with normotensive parents (NN) ($P < 0.05$). (**a**) Results obtained for single nucleotide polymorphism (SNP) rs10951982 located in intron 1. The group of children with GA/AA genotype had lower diastolic blood pressure (DBP) index compared with subjects with GG genotype. (**b**, **c**, and **d**) Results obtained for SNP rs836478 located in intron 3, using the Kruskal–Wallis and Dunn's test ($P < 0.05$). (**b**) The group of NN children with TT genotype had higher plasminogen activator inhibitor-1 levels compared with subjects with TC/CC genotype. The group of NH children with TT genotype had (**c**) higher metalloproteinase-9 levels and (**d**) lower interleukin 6 (IL-6) levels compared with subjects with TC/CC genotype.

extracellular matrix remodeling and thus playing an important role in the pathogenesis of cardiac remodeling.[39]

These results show that in the NH group there is an increased risk of biomarkers (IL-6 and MMP9) associated with the TT genotype of the SNP rs836478. However, there is a discrepancy because the risk genotype is more frequent in NN than NH. This discrepancy could be explained by the presence of other factors—inherited from the subjects' hypertensive parents—that affect the biomarkers levels seen in this cohort.

In summary, we report the frequency and distribution of SNPs rs10951982 (intron 1, G/A) and rs836478 (intron 3, C/T) in the *RAC1* gene in a Spanish-Amerindian pediatric cohort, showing a similar allelic frequency and genotypic distribution as described in the NCBI databases. These SNPs in the *RAC1* gene are associated with some clinical (DBP) and biochemical (IL-6, MMP9, and PAI-1) parameters related to the development and progression of hypertension. However, we cannot rule out the possibility that additional factors, such as environmental or epigenetic factors, are contributing to the association of these polymorphisms with parameters of inflammation or the possibility that these additional factors are more significant. Thus, additional genetic and biochemical studies will be necessary to determine the risks in the phenotype of these subjects that these SNPs may have in later stages of life.

SUPPLEMENTARY MATERIAL

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

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DISCLOSURE

The authors declared no conflict of interest.

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