Different phenotypes of the *NAT2* gene influences hydralazine antihypertensive response in patients with resistant hypertension

Aim: Hydralazine, a vasodilator used in resistant hypertension (RH) treatment is metabolized by an acetylation reaction mediated by *N*-acetyltransferase 2, the activity of which depends on *NAT2* polymorphisms. Our aim was to evaluate whether different acetylation phenotypes influenced the antihypertensive effect of hydralazine in patients with RH. **Patients & methods:** DNA samples from 169 RH patients using hydralazine were genotyped by sequencing the *NAT2* coding region, and acetylation phenotypes were defined. **Results:** Sixty-five patients (38.5%) were intermediate, 60 (35.5%) slow and 21 (12.4%) fast acetylators. Twenty-three (13.6%) patients were indeterminate. Upon association analysis, only slow acetylators had significant blood pressure reductions after hydralazine use, with mean 24-h systolic and diastolic blood pressure reductions of 9.2 and 5.5 mmHg. Four patients presented hydralazine adverse effects resulting in drug withdrawal, three of them were slow acetylators. **Conclusion:** The slow acetylation phenotype, determined by polymorphisms within *NAT2*, influenced both the antihypertensive and adverse effects of hydralazine in RH.

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KEYWORDS: Brazilian population ■ hydralazine ■ *NAT2* polymorphism ■ resistant hypertension ■ SNP

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Hypertension is the leading attributable cause of death worldwide. It is a significant costly and escalating global healthcare problem affecting approximately 1.2 million people, and is associated with an increased risk of myocardial infarction, stroke, heart failure, kidney disease and death. It is a multifactorial disorder influenced by several genetic and epigenetic factors. Different studies have been performed with candidate genes. In particular, polymorphisms of the angiotensinogen gene (M235T), and insertion/deletion of angiotensin-1, were associated with essential arterial hypertension in a Slovene population. Additionally, an association between the -262C/T polymorphism in the catalase gene promoter and the C242T polymorphism of the NADPH oxidase *P22phox* gene and essential arterial hypertension in patients with diabetes mellitus Type 2 has also been reported, indicating the possible implication of the oxidative stress gene NADPH oxidase in the pathogenesis of arterial hypertension in these patients [1,2]. Approximately 5–15% of all patients with high blood pressure (BP) have resistant hypertension (RH) [3–5]. RH is defined as failure to achieve BP targets (i.e., BP <140/90 mmHg in general; and <130/80 mmHg in patients with diabetes mellitus or chronic renal disease) despite the concurrent use of three or more antihypertensive drugs of different classes, including a diuretic, at their optimal doses [3,6]. Cases of pseudoresistance, which may result from poor compliance with treatment, inadequate antihypertensive medication, incorrect BP measurement or the white-coat effect, must be identified. Exaggerated white-coat effect (called white-coat RH) is present when the patient's BP is <140/90 mmHg at the doctor's office or hospital and <135/85 mmHg when measured out of office, preferentially by ambulatory BP monitoring [3,6].

The objective of treating high BP is the prevention of hypertensive end-organ damage and reduction of cardiovascular morbidity and mortality. Control of ambulatory BP is the most important factor in improving cardiovascular prognosis in RH [7]. Its pharmacological treatment usually consists of the use of at least three antihypertensive drugs including a diuretic. Several drugs are commonly used as first-line combinations in RH treatment, including diuretics, β-blockers, angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor antagonists, and calcium channel blockers. As an add-on fourth antihypertensive drug, spironolactone, an aldosterone antagonist diuretic, is often recommended [8].

Future Medicine part of fsg

Hydralazine is a direct-acting vasodilator that has been used for the treatment of hypertension since 1952. Although it has largely been replaced by newer antihypertensive drugs with more acceptable tolerability profiles, hydralazine is still widely used in many countries due its lower cost [9] and particularly in RH, as a fifth drug in antihypertensive regimens. Hydralazine acts by dilating resistance arterioles, thus reducing peripheral resistance. It is primarily metabolized in the liver, by an acetylation reaction, and also forms hydrazones (e.g., the pyruvic acid hydrazone and the acetone hydrazone), which may have some activity in reducing BP. The rate of the *N*-acetylation step has a trimodal distribution and is determined genetically. The acetylation status determines hydralazine systemic biovailability and, because response is determined to a significant extent by serum drug levels, it also determines the drug response [10]. Thus, the acetylation phenotype may influence interindividual variations in hydralazine effectiveness, as well as the incidence of adverse side effects. Indeed, three major genetically determined phenotypes are observed: fast, intermediate and slow acetylators. Hydralazine-induced systemic lupus erythematosus, the most-feared side effect, has mainly been associated with the slow acetylator phenotype [11,12].

Table 1. Frequency of point mutations in the *NAT2* gene among 169 Brazilian patients with resistant hypertension using hydralazine.

SNPs (n = 15)	Alleles (n = 338)	Frequency (%)†	Effect	Amino acid change
29A	01	0.30	Missense	I10T
30A‡	05	1.48	Synonymous	I11I
33A	11	3.25	Synonymous	G12G
191A§	07	2.10	Missense	R64Q
282T§	104	31.0	Synonymous	Y94Y
341C§	124	36.6	Missense	I114T
403G	05	1.48	Missense	L135V
481T§	117	34.6	Synonymous	L161L
590A§	65	19.2	Missense	R197Q
609T	02	0.60	Missense	E203B
766G	03	0.89	Missense	K256E
803G§	144	42.6	Missense	K268R
824C‡	02	0.60	Missense	L274S
838A	02	0.60	Missense	V280M
857A§	13	3.85	Missense	G286E

†*Frequency refers to 338 alleles.*
‡*New SNPs.*
§*Most common SNPs in the world.*

The enzyme arylamine *N*-acetyltransferase 2, coded by the *NAT2* gene, is involved in the human physiological response to a wide range of xenobiotic compounds, including many clinically useful drugs (e.g., hydralazine) and a variety of exogenous chemicals present in the diet and environment. *NAT2* is a highly polymorphic gene and the presence of several SNPs in its coding region can alter its enzymatic activity [13]. Gene mapping studies in humans have demonstrated that *NAT2* is an intronless gene located between 170 and 360 kb at chromosome 8p22, adjacent to clusters of CpG island, and presents a coding region of 873 pb that encodes a 290-amino acid protein [14]. To date, the reference *NAT2*4* allele and 65 other allele variants, combinations of up to four SNPs within the coding region, have been identified and classified in human populations [101]. A total of 40 SNPs have been identified in the *NAT2* coding region, including several rare SNPs [15].

Therefore, the aim of this study was to evaluate whether different acetylating phenotypes predicted by *NAT2* genotypes influence the antihypertensive effect of hydralazine in patients with RH.

Patients & methods

Selection of patients & sample handling

In a case–control epidemiologic study model, 169 consanguineously unrelated (defined by personal history), resistant hypertensive patients using hydralazine from the outpatient hypertension clinic of Clementino Fraga Filho University Hospital were enrolled in this study and stratified in subgroups according to the therapeutic outcomes concerning BP control. All patients had clinical, laboratory and ambulatory BP monitoring (ABPM) data collected at baseline and during follow-up. In total, 61 patients were not using hydralazine at baseline and hence had a before and after hydralazine ABPM. Patients with secondary causes of hypertension were excluded, except sleep apnea. Routinely, primary hyperaldosteronism was defined as a serum aldosterone:renin ratio of >30, renal artery stenosis by duplex scan or renal scintigraphy, and Cushing syndrome by morning serum cortisol >50 nmol/l after a midnight 1-mg dexamethasone suppression. Other secondary causes (pheochromocytoma or thyroid diseases) were investigated whenever there were any clinical signs and/or symptoms. Sleep apnea was not excluded because of its very

high prevalence in patients with RH (nearly 80%). This study was approved by the Ethical Committee of Oswaldo Cruz Foundation and written informed consent was obtained from all of the enrolled patients.

A 5-ml sample of venous blood was collected from each patient and stored at -20°C. Genomic DNA was isolated from 200 µl of frozen whole blood using the QIAamp® DNA Blood Mini Kit (Qiagen Inc., USA), according to the manufacturer's specifications. After extraction, DNA samples were stored at -20°C.

NAT2 genotyping

Genotyping of the *NAT2* coding region was achieved by PCR and sequencing was performed using ABI PRISM Big Dye Terminator v.3.1 kit (PE Applied Biosystems, CA, USA) according to the manufacturer's recommendations on an ABI PRISM 3730 DNA Analyser (PE Applied Biosystems), as previously described [16]. Briefly, an 1093-bp DNA fragment comprising the entire *NAT2* coding region was amplified by PCR and sequenced on both DNA strands using a set of primers for amplification (*NAT2*EF 5-TTAGTCACACGAGGAAATCAAA-3 and *NAT2*ER 5-AAATGCTGACATTTT-TATGGATGA-3) and an additional internal set (*NAT2* IF 5-ACCATTGACGGCAG-GAATTA-3 and *NAT2* IR 5-TGGTCCAG-GTACCAGATTCC-3), designed with Primer3 software (Broad Institute, MA, USA) [102].

Computational analysis

After alignment with the reference sequence AY331807 (GenBank; National Center for Biotechnology Information, MD, USA) [103], sequence data of each sample were analyzed for SNPs identification through use of SeqScape v.2.6 software (PE Applied Biosystem) [104].

Haplotype reconstruction

To determine the haplotype pair of each patient and to define the acetylation status, haplotype reconstruction was performed using the program PHASE v.2.1.1 (University of Chicago, IL, USA) in default model for recombination rare variation [17,18]. Eight independent runs with 1000 interactions, 500 burn-in interactions and a thinning interval of one were performed. The run that showed the maximum consistency across eight runs was chosen. Additionally, the results that applied the PHASE algorithm repeatedly with default and varying values of number of interaction, burn-in interactions and the thinning interval was pursued. All the runs showed consistency across the eight runs chosen.

Table 2. Genotype characterization of *NAT2* gene and determination of acetylation status in 169 Brazilian patients with resistant hypertension using hydralazine.

Cluster designation	Absolute number	Proportion (%)
**5/*5*	24	14.2
**5/*6*	22	13.0
**5/*7*†	02	1.18
**5/*14*†	03	1.78
**6/*6*†	03	1.78
**6/*14*†	02	1.18
**7/*6*†	04	2.38
Total slow acetylation	60	35.5
**4/*4*	13	7.70
**4/*12*†	05	2.95
**4/*13*†	01	0.59
**12/*12*†	01	0.59
**13/*13*†	01	0.59
Total fast acetylation	21	12.4
**4/*5*	21	12.4
**4/*6*	14	8.30
**4/*7*†	05	2.95
**4/*14*†	02	1.18
**5/*12*†	08	4.70
**5/*13*†	08	4.70
**6/*12*†	03	1.78
**6/*13*†	04	2.38
Total intermediate acetylation	65	38.4
Indeterminate acetylation	23	13.6
Total	169	100

†*Genotype frequencies lower than 5%.*

Statistical analysis

Statistical analysis included nonparametric Kruskal–Wallis and χ^2 tests for comparisons among different acetylating subgroups, and Wilcoxon signed rank test and McNemar's test for comparisons of BPs before and after hydralazine use. All analyses were adjusted for multiple comparisons by Bonferroni's correction. Since for each independent variable three comparisons were performed (the three subgroups – slow, rapid and indeterminate acetylator status were compared with the reference intermediate acetylator subgroup), p-values were considered significant if <0.017 (i.e., 0.05 divided by 3). Expected genotype frequencies were calculated

Table 3. Baseline characteristics of all 169 resistant hypertension patients who used hydralazine during follow-up, divided according to *NAT2* acetylation status.

Characteristics	All patients (n = 169)	Intermediate acetylator (n = 65)	Slow acetylator (n = 60)	Rapid acetylator (n = 21)	Indeterminate acetylator (n = 23)
Male (%)	27.8	30.8	23.3	14.3	43.5
Age (years)	64.4 (10.4)	64.9 (9.8)	64.8 (10.4)	61.7 (9.1)	64.9 (12.9)
White ethnicity (%)	51.2	51.6	51.7	52.4	47.8
BMI (kg/m^2)	31.1 (5.8)	30.7 (5.2)	31.5 (6.4)	31.4 (6.7)	31.1 (5.4)
Waist circumference (cm)	101 (12)	100 (11)	101 (11)	103 (13)	101 (15)
Physical inactivity (%)	66.9	64.6	66.7	57.1	82.6
Diabetes (%)	40.2	32.3	46.7	57.1	30.4
Current smoker (%)	12.4	13.8	13.3	4.8	13.0
Dyslipidemia (%)	70.1	57.8	74.6	90.5*	73.9
Clinical target organ damage					
Coronary heart disease (%)	20.1	21.5	25.0	9.5	13.0
Cerebrovascular disease (%)	16.2	12.3	11.7	36.8*	21.7
Peripheral arterial disease (%)	9.5	10.8	10.0	14.3	0
Subclinical target organ damage					
Left ventricular hypertrophy (%)	73.0	72.6	67.2	71.4	90.9
Chronic kidney disease (%)	50.9	46.0	53.3	52.4	56.5
Antihypertensive treatment					
Antihypertensive drugs in use (n)	4 (3–5)	4 (3–5)	4 (3–5)	4 (3–5)	4 (3–5)
ACE inhibitors/AR blockers (%)	93.5	92.3	93.3	90.5	100
β-blockers (%)	81.7	83.1	75.0	90.5	87.0
Calcium channel blockers (%)	57.4	56.9	60.0	52.4	56.5
Hydralazine (%)	63.9	67.7	58.3	57.1	69.6
Central α agonists (%)	17.2	18.5	16.7	19.0	13.0
Systolic BP at baseline (mmHg)					
Clinic	173 (26)	173 (25)	177 (27)	166 (22)	170 (30)
Ambulatory 24 h	137 (18)	135 (17)	140 (17)	137 (21)	134 (19)
Ambulatory daytime	139 (17)	137 (18)	143 (17)	136 (23)	140 (19)
Ambulatory night-time	127 (20)	125 (20)	129 (20)	127 (20)	130 (21)
Diastolic BP at baseline (mmHg)					
Clinic	96 (17)	97 (16)	97 (18)	92 (18)	94 (16)
Ambulatory 24 h	80 (12)	80 (11)	80 (11)	76 (15)	80 (13)
Ambulatory daytime	82 (13)	82 (12)	83 (12)	78 (16)	82 (14)
Ambulatory night-time	73 (13)	73 (13)	72 (12)	69 (14)	74 (14)
Nondipping pattern (%)	55.4	57.1	47.5	61.9	65.2
Uncontrolled ambulatory BP (%)	62.3	56.3	71.2	52.4	65.2
Laboratory variables					
Fasting glycemia (mg/dl)	123 (57)	122 (61)	123 (54)	123 (43)	127 (66)

Values are mean (standard deviation) or proportions, except for number of antihypertensive drugs, serum creatinine, glomerular filtration rate and albuminuria, which are medians (interquartile range).
**p < 0.05 for comparisons with the intermediate acetylators subgroup (the reference group).*
AR: Angiotensin II receptor; BP: Blood pressure; HDL: High-density lipoprotein.

Table 3. Baseline characteristics of all 169 resistant hypertension patients who used hydralazine during follow-up, divided according to *NAT2* acetylation status (cont.).

Characteristics	All patients (n = 169)	Intermediate acetylator (n = 65)	Slow acetylator (n = 60)	Rapid acetylator (n = 21)	Indeterminate acetylator (n = 23)
Laboratory variables (cont.)					
Total cholesterol (mg/dl)	208 (46)	208 (46)	208 (46)	204 (44)	212 (51)
HDL-cholesterol (mg/dl)	46 (13)	47 (15)	47 (12)	44 (10)	46 (15)
Triglycerides (mg/dl)	162 (88)	164 (83)	150 (93)	169 (81)	181 (92)
Serum creatinine (mg/dl)	0.9 (0.8–1.2)	0.9 (0.7–1.2)	0.9 (0.8–1.1)	1.0 (0.8–1.2)	1.0 (0.7–1.3)
Glomerular filtration rate (ml/min/1.73 m^2)	72 (56–90)	74 (58–90)	76 (55–92)	70 (55–87)	70 (55–86)
Albuminuria (mg/24 h)	19 (11–69)	17 (11–57)	18 (11–63)	23 (72–460)	25 (12–80)

Values are mean (standard deviation) or proportions, except for number of antihypertensive drugs, serum creatinine, glomerular filtration rate and albuminuria, which are medians (interquartile range).
**p < 0.05 for comparisons with the intermediate acetylators subgroup (the reference group).*
AR: Angiotensin II receptor; BP: Blood pressure; HDL: High-density lipoprotein.

from respective single allele frequencies according to the Hardy–Weinberg equation. The observed and expected gene frequencies were compared using a χ^2 test for Hardy–Weinberg equilibrium. All statistics were carried out using SPSS statistical package version 19.0.

Results

Genetic variability of the *NAT2* gene in the studied population

Sequence analysis of the *NAT2* from 169 patients enrolled in this study revealed the presence of 15 different SNPs, 13 already described and registered in the official site of *NAT2* nomenclature [101] and two new SNPs located at positions 30 and 824 (30T>A and 824T>C). The SNP at position 30 is synonymous, while the 824T>C is a nonsynonymous one leading to an amino acid change from the leucine residue to serine at codon 274 (Leu274Ser). Seven of the identified SNPs represent the most frequently found *NAT2* SNPs among different populations. Table 1 shows the frequency of each SNP variant, the effect on protein sequence and the amino acid changes.

Haplotype reconstruction, allele characterization & phenotype determination

After haplotype pair reconstruction, 30 *NAT2* alleles were characterized, from which 11 were new. After genotype determination, patients were classified as intermediate, slow and fast acetylators. Table 2 shows the selected haplotypes, allele frequencies, as well the acetylation phenotypes. From a total of 169 patients, 65 (38.5%; 95% CI: 30.2–49.0%) were classified as intermediate, 60 (35.5%; 95% CI: 27.6–45.7%) as slow and 21 (12.4%; 95% CI: 8.1–19.0%) as fast acetylators. From the remaining 23 patients (13.6%; 95% CI: 9.0–20.5%) no characterization of the acetylation phenotype was achieved.

Association between NAT2 acetylating phenotypes & BP reduction

Among the 169 patients enrolled in this study, baseline clinical, laboratory and BPs were identical among the subgroups, except that rapid acetylators had a higher prevalence of dyslipidemia and of cerebrovascular disease than the other subgroups (Table 3). Sixty-one patients were not using hydralazine at baseline and hence had a before and after hydralazine ABPM. The median dose of hydralazine was 150 mg/day (range: 50–300 mg/day). Table 4 shows clinic and ambulatory BP changes after hydralazine treatment. Significant BP reductions were observed only in the slow acetylation subgroup. Mean 24-h systolic and diastolic BP reductions were 9.2 mmHg (95% CI: 2.4–15.9 mmHg) and 5.5 mmHg (95% CI: 1.3–9.8 mmHg), respectively; whereas mean clinic BP reductions were 18.6 mmHg (95% CI: 2.5–34.7 mmHg) and 8.8 mmHg (95% CI: 0.6–17.1 mmHg), respectively. In slow acetylators, hydralazine equally reduced both daytime and night-time BPs. Figures 1 & 2 show clinic and ambulatory BP changes after hydralazine treatment. Four patients presented hydralazine adverse effects resulting in drug withdrawal, three of them were slow acetylators (one lupus-like syndrome).

Discussion

Currently, the influence of genetic variations on interindividual differences in pharmacological or toxicological response to drugs is

well established. Pharmacogenetics/genomics are fields of growing importance in the postgenome era raising the possibility of customized therapy for different individuals. Pharmacogenetic studies of arylamine *N*-acetyltransferase genes (*NAT1* and *NAT2*) were pioneering and variation in NAT activity was one of the earliest pharmacogenetic traits to be recognized [19]. Genetic polymorphisms within the *NAT2* locus, giving rise to either 'slow' or 'fast' acetylation phenotypes, are associated with susceptibility to several diseases, such as different types of cancer and psoriasis [20,21], and, in parallel, are also involved with response to environmental toxins and differences in therapeutic outcomes upon treatment with NAT2-metabolized drugs (e.g., dapsone, isoniazid and hydralazine). Genotype and phenotype correlation is well defined and the precise functional effects of most SNPs on acetylation are being characterized. The four common SNPs conferring an amino acid change to produce slow acetylating phenotypes are rs1801279 (191G>A; Arg64Gln) in *NAT2*14,* rs1801280 (341T>C; Ile114Thr) in *NAT2*5, NAT2*14C* and *NAT2*14F,* rs17799930 (590G>A; Arg197Gln) in *NAT2*6, NAT2*5E* and *NAT2*14D,* and rs1799931 (857G>A; Gly286Glu) in *NAT2*7.* However, there are significant differences between interethnic populations in *NAT2* allele distribution and frequency. For example, the slow acetylating allele *NAT2*5,* which is commonly found in Europeans and Africans, is extremely rare in Japanese, Koreans and Taiwanese. In our study we found 15 SNPs within the *NAT2* coding region: rs72466456 (29C>T, 30T>A and 33C>A); rs1801279 (191G>A); rs1041983 (282C>T); rs1801280 (341T>C and 403C>G); rs1799929 (481C>T);

Table 4. Blood pressures in 61 resistant hypertensive patients who initiated hydralazine during follow-up, divided according to *NAT2* acetylation status.

BP	Hydralazine use	Intermediate acetylator (n = 21)	Slow acetylator (n = 24)	Rapid acetylator (n = 9)	Indeterminate acetylator (n = 7)
Systolic BP (mmHg)					
Clinic	Before	176 (28)	178 (33)	174 (34)	174 (35)
	After	165 (23)	163(25)*	168 (34)	159 (22)
Ambulatory 24 h	Before	138 (15)	143 (15)	142 (13)	144 (13)
	After	144 (18)	134 (17)*	139 (18)	152 (23)
Ambulatory daytime	Before	140 (16)	146 (14)	144 (14)	146 (13)
	After	147 (19)	136 (17)*	141 (18)	155 (26)
Ambulatory night-time	Before	131 (15)	135 (17)	133 (13)	137 (18)
	After	133 (19)	126 (21)*	130 (16)	140 (14)
Diastolic BP (mmHg)					
Clinic	Before	96 (18)	94 (19)	86 (18)	91 (15)
	After	88 (11)	87 (18)*	83 (21)	86 (11)
Ambulatory 24 h	Before	81 (10)	80 (9)	74 (12)	83 (10)
	After	83 (14)	75 (11)**	75 (12)	87 (12)
Ambulatory daytime	Before	83 (11)	82 (10)	77 (12)	85 (10)
	After	86 (14)	76 (11)**	77 (12)	88 (14)
Ambulatory night-time	Before	75 (10)	74 (9)	67 (14)	79 (14)
	After	76 (16)	69 (11)*	68 (11)	80 (10)
Nondipping pattern (%)	Before	61.9	70.8	66.7	71.4
	After	47.6	54.2	66.7	57.1
Uncontrolled daytime BP (%)	Before	61.9	79.2	77.8	85.7
	After	76.2	37.5**	66.7	85.7

Values are mean (standard deviation) or proportions.
**p < 0.05 for comparisons before and after hydralazine use (Wilcoxon signed rank test and McNemar's test), after Bonferroni's correction for multiple comparisons.*
***p < 0.05 for comparisons among groups of different NAT2 metabolizers (Kruskal–Wallis test and χ^2 test, with the intermediate metabolizer group as the reference), after Bonferroni's correction for multiple comparisons.*
BP: Blood pressure.

rs1799930 (590G>A, 609G>T and 766A>G); rs1208 (803A>G, 824T>C and 838G>A); rs1799931 (857G>A), of which seven are the most frequent SNPs in the world and two, at positions 30T>A (silent) and 824T>C (nonsynonymous L274S), are new SNPs. After haplotype characterization, the most frequent allele was the *NAT2*5B* (28%), which carries the nonsynonymous mutation rs1801280 341T>C resulting in one of the most altered isoforms of NAT2 in comparison with the other slow isoforms. The *NAT2*6A* allele, which carries the nonsynonymous mutation rs1799930 590G>A, was present in 16.3%; while *NAT2*7B*, carrying the nonsynonymous mutation rs1799931 857G>A, was present in 3.5% of the whole alleles found, mainly restricted to the Brazilian population from Rio de Janeiro. Regarding the alleles associated with fast acetylation, the *NAT2*4* allele, which is considered to be the wild-type and presenting no polymorphism, was found in 23% of our cohort. Finally, the distribution of the 'intermediate' phenotype, which results from heterozygous fast/slow alleles, was found in 65 patients (38.5%).

Concerning the interethnic distribution of *NAT2* SNPs, alleles and phenotypes, our findings are in accordance with a previous study from our group that compared *NAT2* alleles found in another cohort from Rio de Janeiro with the ones found in Europeans, Asians, Amerindians and African populations, in which significant differences were observed [16]. Several studies suggest that these differences in phenotype patterns depend, at least in part, on lifestyle [22]. Recent genomic studies have provided growing evidence that cultural processes can have a profound impact on the human genome, triggering significant changes in allele frequencies in response to culturally modified environmental conditions [23]. In addition, the genetic diversity of *NAT2* is structured in the human species, the major differences in allele frequencies exist between populations from different ethnic or geographic origins, but most genetic diversity occurs between individuals and this is the most important aspect, concerning both therapeutic and epidemiological applications [24,25].

Hydralazine is indicated for long-term therapy of essential hypertension, particularly in treatment-resistant patients, for short-term therapy of pregnancy-induced hypertension and eclampsia, and in the therapy of hypertensive crisis. The association between hydralazine response and acetylation status has already been previously reported [10], but, to our knowledge, our study was the first to evaluate it in patients with RH, an important subgroup of general hypertensives in whom hydralazine treatment is particularly indicated.

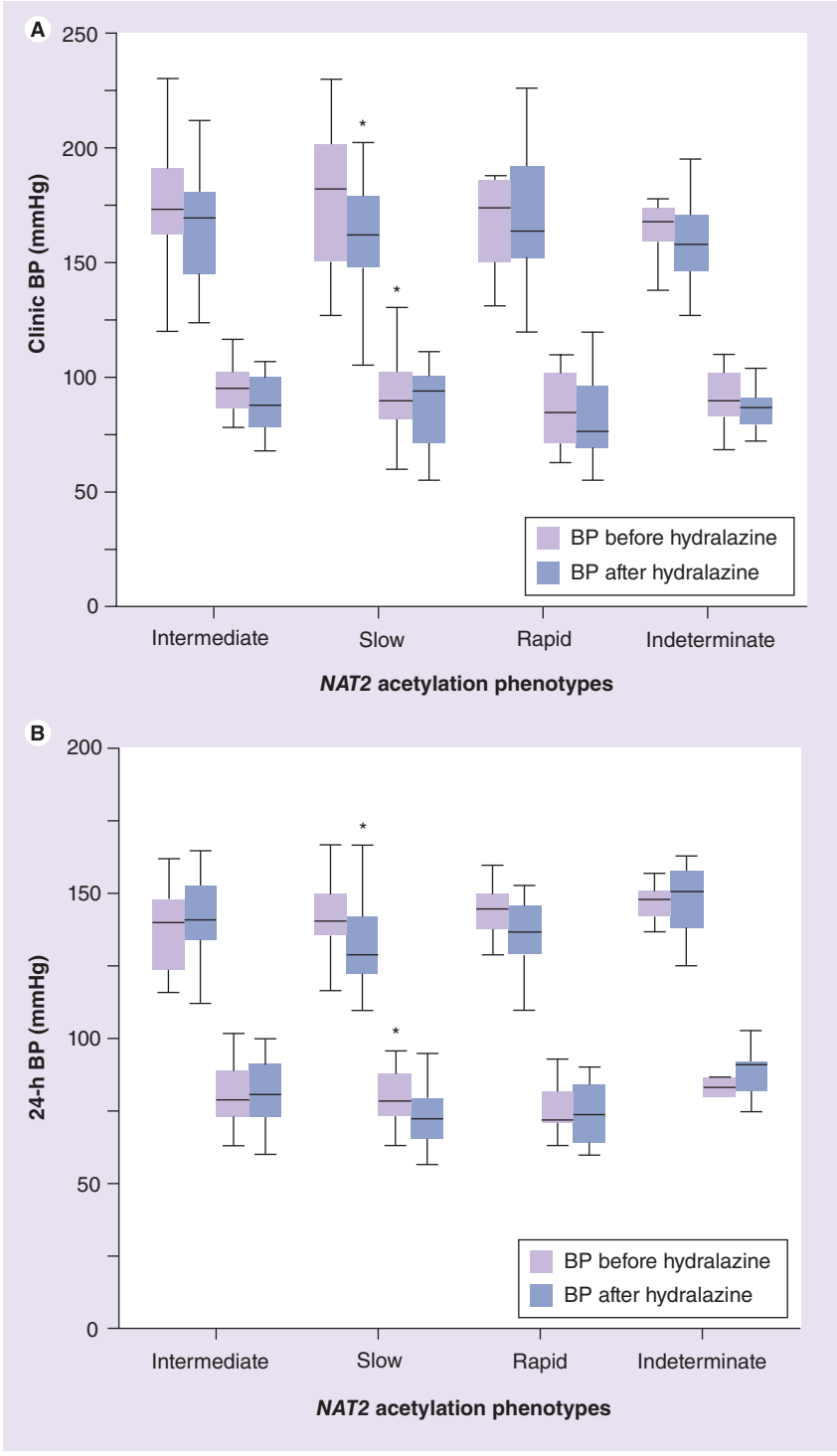
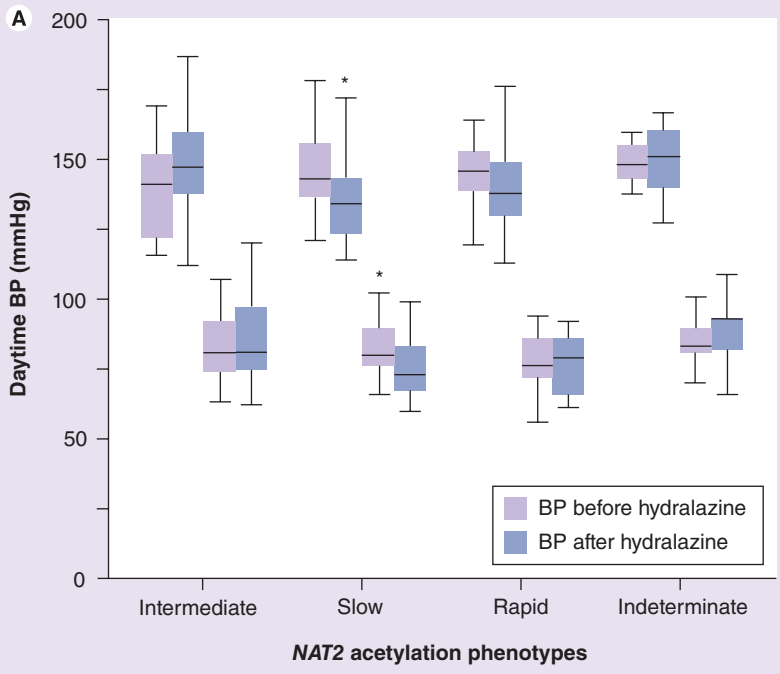


Figure 1. 24-h blood pressures before and after hydralazine use in resistant hypertensive patient groups. (A) Clinic and **(B)** ambulatory 24-h BPs before (purple boxes) and after (blue boxes) hydralazine use in resistant hypertensive patients grouped according to *NAT2* acetylation phenotypes.
*$p < 0.05$.
BP: Blood pressure.



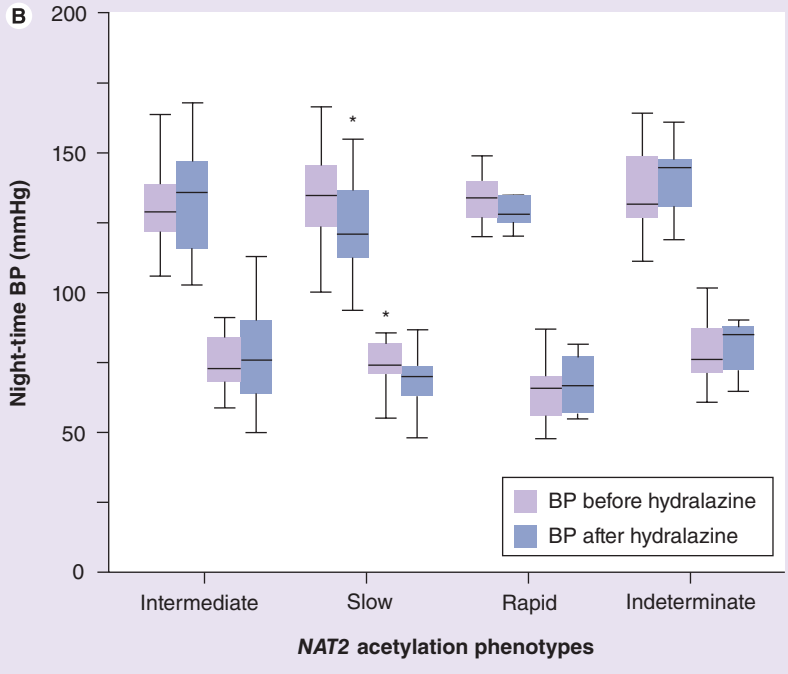


Figure 2. Ambulatory daytime and night-time blood pressures before and after hydralazine use in resistant hypertensive patient groups.
(A) Ambulatory daytime and **(B)** night-time blood pressures before (purple boxes) and after (blue boxes) hydralazine use in resistant hypertensive patients grouped according to *NAT2* gene phenotypes of acetylation.
*$p < 0.05$.
BP: Blood pressure.

Hydralazine-induced lupus has been reported frequently and is a serious adverse reaction. The incidence is dose dependent, with 5.4% of patients developing hydralazine-induced lupus with 100 mg daily and 10.4% with 200 mg daily after 3 years of treatment in patients with the slow acetylating phenotype [26]. Drug-induced lupus erythematosus is defined as a lupus-like syndrome temporally related to continuous drug exposure, which resolves after discontinuation of the offending drug. There are currently no standard diagnostic criteria for drug-induced lupus erythematosus and the pathologic mechanisms are still unclear. Although hydralazine-induced lupus was thought originally to occur only in slow acetylators, it does occasionally occur in fast acetylators.

Our results suggest that *NAT2* is the main genetic component responsible by the antihypertensive effect of hydralazine in RH. Only the slow acetylators had significant BP reductions after hydralazine use, although they also had higher incidence of adverse side effects. Patients with RH had a general poor antihypertensive response to drug treatment, moreover when an antihypertensive drug is added on a background of several other antihypertensive drugs already in use, as is the case for hydralazine that is usually used as a fourth or fifth drug in an antihypertensive regimen. That is why only the slow acetylators, inducing higher serum levels of the drug, presented a consistent antihypertensive effect.

Concerning the study limitations, we understand that the relatively small sample size (169 patients), although quite large for a study with resistant hypertensives, should be higher to increase the statistical power for a more comprehensive evaluation. Another issue is that no direct functional evaluation was performed, such as pharmacokinetic assays to detect hydralazine metabolites (e.g., the pyruvic acid and acetone hydrazones) in the urine of slow acetylators and comparison with different genetic profiles of acetylation. However, we used the BP response to hydralazine, evaluated by a confident method such as 24-h ambulatory BP monitoring [27], as the surrogate to detect different drug serum levels, as the antihypertensive effect of hydralazine is directly related to its serum levels [10]. Finally, although measurement of the acetylation phenotype is rather simple, it is not readily available to general practitioners. The safety of long-term hydralazine treatment is clearly related to the individual drug doses and acetylation status. In this study, *NAT2* genotyping was performed by sequencing, overcoming the possible problems of misclassification of the acetylation status.

Conclusion & future perspective

Genotyping interindividual variability at the *NAT2* locus may not only help the identification

of the best resistant hypertensive candidates to hydralazine treatment (the slow acetylators), but also the determination of the most suitable dosage for individual patients, possibly reducing adverse side effects, and the need for excessive outpatient visits and hospitalizations. Future studies should focus on larger samples of resistant hypertensive patients and also on cost-effective analysis of implementing treatment strategies of RH that takes into account pharmacogenetic evaluation of *NAT2* polymorphisms.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Hydralazine is an antihypertensive medication mainly used as an add-on fourth or fifth drug in a resistant hypertension treatment regimen. Its efficacy may depend on *NAT2* gene polymorphisms that regulate metabolizing acetylation phenotypes; however, it has never been evaluated in resistant hypertensive patients.
- In 169 resistant hypertensive individuals, *NAT2* genotyping showed that 38.4% were intermediate, 35.5% were slow and 12.4% were fast acetylators, while 13.6% had an indeterminate acetylation status.
- On 24-h ambulatory blood pressure monitoring, only the slow acetylators had significant blood pressure reductions after hydralazine use, although they also had a higher incidence of adverse side effects.
- *NAT2* genotyping may help to identify resistant hypertensive patients who would best benefit from hydralazine treatment.

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