NR3C1 polymorphisms in Brazilians of Caucasian, African, and Asian ancestry: glucocorticoid sensitivity and genotype association

Polimorfismos NR3C1 *em brasileiros de ascendência caucasiana, africana e asiática: sensibilidade aos glicocorticoides e associação de genótipos*

Manoel Carlos L. A. Souza[1], Clarissa S. Martins[1], Ivan M. Silva-Junior[1], Rosangela S. Chriguer[1], Ana C. Bueno[2], Sonir R. Antonini[2], Wilson Araújo Silva Jr.[3,4], Marco A. Zago[1], Ayrton C. Moreira[1], Margaret de Castro[1]

ABSTRACT

Objective: The Brazilian population has heterogeneous ethnicity. No previous study evaluated *NR3C1* polymorphisms in a Brazilian healthy population. **Materials and methods:** We assessed *NR3C1* polymorphisms in Brazilians of Caucasian, African and Asian ancestry (n = 380). In a subgroup (n = 40), we compared the genotypes to glucocorticoid (GC) sensitivity, which was previously evaluated by plasma (PF) and salivary (SF) cortisol after dexamethasone (DEX) suppression tests, GC receptor binding affinity (K_d), and DEX-50% inhibition (IC_{50}) of concanavalin-A-stimulated mononuclear cell proliferation. p.N363S (rs6195), p.ER22/23EK (rs6189-6190), and *BclI* (rs41423247) allelic discrimination was performed by Real-Time PCR (Polymerase Chain Reaction). Exons 3 to 9 and exon/intron boundaries were amplified by PCR and sequenced. **Results:** Genotypic frequencies (%) were: rs6195 (n = 380; AA:96.6/AG:3.14/GG:0.26), rs6189-6190 (n = 264; GG:99.6/GA:0.4), rs41423247 (n = 264; CC:57.9/CG:34.1/GG:8.0), rs6188 (n = 155; GG:69.6/GT:25.7/TT:4.7), rs258751 (n = 150; CC:88.0/CT:10.7/TT:1.3), rs6196 (n = 176; TT:77.2/TC:20.4/CC:2.4), rs67300719 (n = 137; CC:99.3/CT:0.7), and rs72542757 (n = 137; CC:99.3/CG:0.7). The rs67300719 and rs72542757 were found only in Asian descendants, in whom p.N363S and p.ER22/23EK were absent. The p.ER22/23EK was observed exclusively in Caucasian descendants. Hardy-Weinberg equilibrium was observed, except in the Asian for rs6188 and rs258751, and in the African for p.N363S. The K_d, IC_{50}, baseline and after DEX PF or SF did not differ between genotype groups. However, the mean DEX dose that suppressed PF or SF differed among the *BclI* genotypes ($P = 0.03$). DEX dose was higher in GG- (0.7 ± 0.2 mg) compared to GC- (0.47 ± 0.2 mg) and CC-carriers (0.47 ± 0.1 mg). **Conclusion:** The genotypic frequencies of *NR3C1* polymorphisms in Brazilians are similar to worldwide populations. Additionally, the *BclI* polymorphism was associated with altered pituitary-adrenal axis GC sensitivity. Arq Bras Endocrinol Metab. 2014;58(1):53-61

Keywords

NR3C1 genotypes; allelic frequencies; glucocorticoid sensitivity

[1] Department of Internal Medicine, School of Medicine of Ribeirao Preto, Universidade de Sao Paulo (FMRP-USP), Ribeirao Preto, SP, Brazil
[2] Department of Pediatrics, FMRP-USP, Ribeirao Preto, SP, Brazil
[3] Department of Genetics, FMRP-USP, Ribeirao Preto, SP, Brazil
[4] Center of Medical Genomics, FMRP-USP, Ribeirao Preto, SP, Brazil

RESUMO

Objetivo: Este estudo avalia polimorfismos (SNPs) do *NR3C1* na população brasileira, que possui origem étnica heterogênea. **Materiais e métodos:** SNPs do *NR3C1* foram avaliados em brasileiros de ancestralidade caucasiana, africana ou japonesa (n = 380). Em um subgrupo (n = 40), os genótipos foram comparados à sensibilidade aos glicocorticoides (GC), previamente avaliada por cortisol plasmático (PF) e salivar (SF) após supressão com dexametasona (DEX), ensaio de afinidade do receptor ao GC (K_d) e inibição por DEX de 50% da proliferação de mononucleares estimulada por concanavalina-A (IC_{50}). Discriminação alélica de p.N363S (rs6195), p.ER22/23EK (rs6189-6190) e *BclI* (rs41423247) foi realizada por PCR em tempo real. Éxons 3 a 9 e transições éxon/íntron foram amplificados e sequenciados. **Resultados:** Frequências genotípicas (%) foram: rs6195 (n = 380; AA:96,6/AG:3,14/GG:0,26), rs6189-6190 (n = 264; GG:99,6/GA:0,4), rs41423247 (n = 264; CC:57,9/CG:34,1/GG:8,0), rs6188 (n = 155; GG:69,6/GT:25,7/TT:4,7), rs258751 (n = 150; CC:88,0/CT:10,7/TT:1,3), rs6196 (n = 176; TT:77,2/TC:20,4/CC:2,4), rs67300719 (n = 137; CC:99,3/CT:0,7), e rs72542757 (n = 137; CC:99,3/CG:0,7). Enquanto rs67300719 e rs72542757 foram exclusivos dos nipodescendentes, p.N363S e p.ER22/23EK estavam ausentes nesses indivíduos. p.ER22/23EK foi exclusivo dos descendentes de caucasianos. Equilíbrio de Hardy-Weinberg foi observado, exceto nos nipodescendentes para rs6188 e rs258751 e nos afrodescendentes para p.N363S. K_d, IC_{50}, PF ou SF basal ou após DEX foram semelhantes entre os genótipos. Entretanto, a dose média de DEX que suprimiu PF ou SF diferiu entre os genótipos *BclI* (P = 0,03), sendo maior nos carreadores GG (0,7 ± 0,2 mg) comparada aos GC (0,47 ± 0,2 mg) e CC (0,47 ± 0,1 mg). **Conclusão:** As frequências genotípicas dos SNPS de *NR3C1* na população brasileira são semelhantes a outras populações. *BclI* foi associado à alteração da sensibilidade ao GC no eixo hipófise-adrenal. Arq Bras Endocrinol Metab. 2014;58(1):53-61

Descritores

Genótipos do *NR3C1*; frequência alélica; sensibilidade aos glicocorticoides

Correspondence to:
Margaret de Castro
School of Medicine of Ribeirao Preto, Universidade de Sao Paulo
Av. dos Bandeirantes, 3900
14049-900 – Ribeirao Preto, SP, Brazil
castrom@fmrp.usp.br

Received on July/4/2013
Accepted on Aug/27/2013

INTRODUCTION

Glucocorticoids (GCs) affect a variety of functions throughout the body, such as glucose and fat metabolism, stress response, immune, and central nervous system activity, cell development and differentiation. Regulation of serum GC concentrations is under the influence of the hypothalamus-pituitary-adrenal (HPA) axis, and circulating GCs themselves exert a negative feedback on hypothalamic and pituitary levels. GCs act via the cytoplasmic glucocorticoid receptor (GR), which is a member of the nuclear receptor family (1,2).

The GR gene (*NR3C1*) maps to chromosome 5q31 (3), and consists of 9 exons (4). The complexity of GR action is the existence of multiple receptor subtypes and isoforms with multiple biological roles (5). Alternative splicing of the GR primary transcript produces two receptor isoforms, GRα and GRβ, which differ at their carboxyl termini. This difference renders GRβ unable to bind GCs (2). GRβ has dominant-negative effects on hormone-induced GRα action (6,7), and an intrinsic transcriptional activity has been recently proposed (8). The GRα/GRβ ratio, however, does not seem to be responsible for interindividual variability of glucocorticoid sensitivity in normal subjects (9). Additionally, each isoform is subject to several post-translational modifications, including phosphorylation, ubiquitinationm and sumoylation, which have been shown to modulate the receptor protein stability and/or function (10).

Numerous *NR3C1* mutations affecting receptor binding, structure, and transcriptional activity have been described (11), especially in patients with rare familial glucocorticoid resistance (11).

Variability in glucocorticoid sensitivity can be observed in several conditions, such as chronic inflammatory and autoimmune diseases, including rheumatoid arthritis (12), pemphigus (13), idiopathic nephrotic syndrome (14), dialysis patients (15), and asthma (16). In addition, there is a considerable variability in the sensitivity to glucocorticoids across individuals (17,18) and some of these differences have been related to polymorphisms, some of which relatively frequent in the GR gene (19-22).

The ethnic origin of the Brazilian population is heterogeneous and unevenly distributed among the states throughout the country, with high degree of miscegenation. In addition to the native Indians and Portuguese colonists, Brazil received immigrants from Africa and the Middle East, and from several other countries including Italy, Spain, Germany, and Japan. However, little information on single nucleotide polymorphisms (SNPs) in the GR gene is available for the Brazilian population. In the present study, we perform an extensive DNA sequence analysis on *NR3C1* in subsets of three different Brazilian populations: Caucasian, African, and Asian descendants to assess the allelic and genotypic frequency of GR SNPs. In addition, we present evidence that GR *BclI* polymorphism is associated with altered sensitivity to GCs.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Institution Ethics Committee (Process nº 25000.136613/2001-02), and informed consent was obtained from all subjects. The sample included Afro-Brazilians based on criteria of physical aspects and of being a descendant from African parents and grandparents; Caucasian individuals were first or second generation of European immigrants mainly from the west and south of Europe; and Japanese descendants included the first or second generation of Japanese immigrants, who came to Brazil in the beginning of 20th century after the First World War. Among these subjects, there were forty healthy individuals of Caucasian (n = 39) and Asian (n = 01) ancestry (19 males and 21 females ranging in age from 22 to 42 years) who had been previously studied in three bioassays that evaluated glucocorticoid sensitivity: plasma and salivary cortisol measurements by radioimmunoassay after overnight 0.25, 0.5 and 1.0 mg dexamethasone (DEX) suppression tests, DEX-mediated 50% inhibition (IC_{50}) of concanavalin-A-stimulated peripheral blood mononuclear cell proliferation (PBMC), and number of binding sites (Bmax) and affinity of glucocorticoid receptor (Kd) (18). Dexamethasone levels were measured by radioimmunoassay using polyclonal antiserum produced in rabbits immunized with dexamethasone 21-acetate (Sigma, ST. Louis, MO, USA) and [^{3}H]-Dexamethasone (Amersham, Little Chalfont, UK) (18). At the time of the study, body weight and height of the subjects were determined, and the body mass index (BMI; mean ± standard error) was 23.2 ± 4.5 kg/m^2.

Genetic analysis

The p.N363S (rs6195), p.ER22/23EK (rs6189 – rs6190), and *BclI* (rs41423247) were genotyped using TaqMan® real-time PCR (Applied Biosystems, Foster City, CA, USA) allelic discrimination technology (Table 1). The p.N363S (rs6195) was evaluated in 380 individuals, including 276 of Caucasian origin, 70 Afro-Brazilians and 34 Asian descendents. The p.ER22/23EK (rs6189 – rs6190), and *BclI* (rs41423247) were evaluated in 264 individuals, including 230 of Caucasian origin, 31 Afro-Brazilians and only 3 Asian descendants. Exons 3 to 9 and intron/exon boundary regions were amplified in 137 to 176 subjects divided in Caucasian, African, and Asian ancestry by PCR using specific primers previously described (23). PCR reactions were followed by automated direct sequencing performed using a commercial kit (ABI Prism® Big Dye™ Terminator Cycle Sequencing Ready Reaction kit; Applied Biosystems, Foster City, CA, USA). The obtained DNA sequences were compared with the *NR3C1* sequence (GenBank accession numbers: NT 029289 GI: 37550092) using the Phred/Phrap/Consed Program (University of Washington, Department of Genome Sciences http://droog.mbt.washington.edu.) to assess GR polymorphisms or mutations.

Statistical analysis

Results are presented as means ± standard deviation (SD). Statistics were carried out using the Mann-Whitney test for continuous variables. To test for differences between categorized variables and genotypes, Pearson Chi-square test was used. Hardy-Weinberg equilibrium was analyzed for all the detected polymorphisms. One-way analyses of variance (ANOVAs) were used to evaluate the differences in all tests of glucocorticoid sensitivity between genotypes. A nonlinear sigmoidal dose-response regression (GraphPad Prism) was performed in a previous dose-response study (18) to obtain more estimated individual values of the DEX dose needed to obtain the cutoff values of plasma cortisol of 50 nmol/liter and salivary cortisol of 2.6 nmol/liter. Spearman's rank correlation coefficient was used to assess the correlation between individual DEX suppressive dose and BMI. Data were analyzed using GraphPad Prism version 4.0 software (GraphPad Software, Inc., La Jolla, CA). Differences were considered significant at $P < 0.05$.

RESULTS

Table 2 shows genotypic and allelic frequencies of polymorphisms detected in the *NR3C1* gene in Caucasian, African, and Asian Brazilian subpopulations. The p.N363S (rs6195) polymorphism, also named rs56149945, an ATT to GTT missense alteration within exon 2, codon 363, that results in a receptor variant with an asparagine to serine substitution in a modulatory region of the *NR3C1* gene, was detected in a frequency (%) of AA:96.6/AG:3.14/GG:0.26 (n = 380).

The p.ER22/23EK (rs6189-6190), a polymorphism described in exon 2, which comprises two point mutations in codons 22 (GAG to GAA change, both encoding for glutamic acid) and 23 (AGG to AAG resulting in an amino acid change from arginine to lysine), was detected in a frequency of GG:99.6/GA:0.4% (n = 264).

The genotypic frequency (%) of the *BclI* (rs41423247) polymorphism, C/G single nucleotide polymorphism in intron 2 of the GR gene, which is 646 nucleotides downstream from exon 2, was CC:57.9/CG:34.1/GG:8.0 (n = 264).

In the Asian descendants, the p.N363S and p.ER22/23EK variants were not found. In fact, p.ER22/23EK polymorphism was observed exclusively in Caucasian descendants. The Hardy-Weinberg equilibrium was not observed in the African subpopulation for p.N363S.

Table 1. Primers and probes used for allelic discrimination

		Primers		Probes
Bcl I	FWD	5'-CAGGGTTCTTGCCATAAAGTAGACA-3'	WT	5'-CTCTTAAAGAGATT**G**ATCAGC-3'
	REW	5'-GCACCATGTTGACACCAATTCC-3'	VT	5'-CTCTTAAAGAGATT**C**ATCAGC-3'
N363S	FWD	5'-GTCATTCCACCAATTCCCGTTG-3'	WT	5'-ACCTATTCCAA**T**TTTCGG-3'
	REW	5'-GTCAAGTTGTCATCTCCAGATCCTT-3'	VT	5'-CCTATTCCAA**C**TTTCGG-3'
ER22/23EK	FWD	5'-AGAAGAAAACCCCAGCAGTGT-3'	WT	5'-CACATCTCCC**C**T**C**TCCTGA-3'
	REW	5'-CAGTAGCTCCTCCTCTTAGGGTTTTA-3'	VT	5'-CACATCTCCC**TT**TCCTGA-3'

REW: reverse; FWD: forward; WT: wild type; VT: variant.

Table 2. NR3C1 polymorphisms, genotypic and allelic frequencies (%) in Caucasian-, African- and Asian-Brazilian subpopulations

Location	db SNP Accession	Minor change	Rare allele	Caucasian n (Genotypic frequency) (allelic frequency)	African n (Genotypic frequency) (allelic frequency)	Asian n (Genotypic frequency) (allelic frequency)	Total n (Genotypic frequency) (allelic frequency)
Exon 2	rs 6195 (p.N363S)	A/G	G	**276** (AA:96/AG:4) (A:98.0/G:2.0)	**70** (AA:97.2/AG:1.4/GG:1.4) (A:97.8/G:2.2)	**34** (AA:100) (A:100)	**380** (AA:96.6/AG:3.14/GG:0.26) (A:98.15/G:1.85)
Exon 2	rs 6189/6190 (p.ER22/23EK)	G/A	A	**230** (GG:99.5/GA:0.5) (G:97.8/A:2.2)	**31** (GG:100) (G:100)	**3** (GG:100) (G:100)	**264** (GG:99.6/GA:0.4) (G:99.8/A:0.2)
Intron 2	rs41423247 *BclI*	C/G	G	**230** (CC:59.5/CG:32.6/GG:7.8) (C:75.8/G:24.2)	**31** (CC:48.3/CG:45.1/GG:6.4) (C:71.0/G:29.0)	**3** (CC:33/CG:33/GG:33) (C:50.0/G:50.0)	**264** (CC:57.9/CG:34.1/GG:8.0) (C:75.0/G:25.0)
Intron D	rs 6188 (IVS4 -16)	G/T	T	**91** (GG:68.1/GT:29.6/TT:2.3) (G:83.0/T:17.0)	**31** (GG:54.8/GT:32.2/TT:13) (G:71.0/T:29.0)	**33** (GG:87.8/GT:9/TT:3.2) (G:92.5/T:7.5)	**155** (GG:69.6/GT:25.7/TT:4.7) (G:82.6/T:17.4)
Exon 8	rs 258751 (p.D678D)	C/T	T	**50** (CC:98/CT:2) (C:99.0/T:1.0)	**50** (CC:82/CT:18) (C:91.0/CT:9.0)	**50** (CC:84/CT:12/TT:4) (C:90.0/T:10.0)	**150** (CC:88/CT:10.7/TT:1.3) (C:93.3/T:6.7)
Intron H	rs72542757 (IVS8 -9)	C/G	G	**48** (CC:100) (C:100)	**40** (CC:100) (C:100)	**49** (CC:98/CG:2) (C:99.0/G:1.0)	**137** (CC:99.3/CG:0.7) (C:99.7/G:0.3)
Exon 9	rs67300719 p.(P750P)	C/T	T	**48** (CC:100) (C:100)	**40** (CC:100) (C:100)	**49** (CC:98/CT:2) (C:99.0/T:1.0)	**137** (CC:99.3/CT:0.7) (C:99.7/T:0.3)
Exon 9	rs 6196 p.(N766N)	T/C	C	**86** (TT:80.2/TC:17.4/CC:2.4) (T:89.0/C:11.0)	**40** (TT:62.5/TC:32.5/CC:5) (T :78.75/C:21.25)	**50** (TT:84/TC:16) (T:92.0/C :8.0)	**176** (TT:77.2/TC:20.4/CC:2.4) (T:87.5/C:12.5)

We also detected, in the Brazilian population, the G>T changing, at intron D/exon5 (rs6188) in a genotypic frequency (%) of GG:69.6/GT:25.7/TT:4.7 (n = 155), and the p.D678D polymorphism (rs258751), at exon 8, in a frequency of CC:88.0/CT:10.7/TT:1.3 (n = 150). For these polymorphisms, the African and Caucasian subpopulations were in Hardy-Weinberg equilibrium, but not the Asian subpopulation.

In exon 9, we detected three polymorphisms: p.N766N polymorphism (rs6196) (n = 177; TT:77.2/TC:20.4/CC:2.4); IVSH -9C>G (rs72542757), a C>G substitution, located in the 9 base pairs before the end of intron H (n = 137; CC:99.3/CG:0.7); and a heterozygous p.P750P polymorphism (rs67300719) (n = 137; CC:99.3/CT:0.7). The variant alleles for rs72542757 and rs67300719 were found only in the Japanese descendants. No polymorphism was detected in exons 3, 4, 6, and 7.

In a previous study, we evaluated glucocorticoid sensitivity by plasma and salivary cortisol measurements after overnight 0.25, 0.5 and 1.0 mg DEX suppression doses. The administration of increasing doses of DEX (0.25, 0.5, and 1.0 mg) resulted in a dose-dependent increase in mean circulating DEX levels (1.4 ± 0.1, 2.6 ± 0.2, and 5.8 ± 0.4 nmol/liter, respectively) (18). Currently, the *NR3C1* polymorphisms is being assessed in those subjects. At baseline and after administration of all three different doses of DEX, no differences in early morning serum or salivary cortisol concentrations were found between *BclI* genotype groups. However, the mean values of DEX dose that suppressed plasma and salivary cortisol (cutoff levels of plasma cortisol was < 50 nmol/L or 1.8 µg/dL and salivary cortisol was 2.6 nmol/L or 92 ng/dL) differed among the three groups (P = 0.03). DEX dose was higher in homozygous G-allele carriers (0.7 ± 0.2 mg), compared with heterozygous (0.47 ± 0.2 mg; P = 0.04) and also with CC-carriers (0.47 ± 0.1 mg; P = 0.03) (Figure 1). There was no correlation between the values of DEX dose that suppressed cortisol and BMI (r = 0.14).

At baseline and after administration of all three different doses of DEX, no differences in early morning serum or salivary cortisol concentrations were found between p.N363S (rs6195), intron D/exon 5 (rs6188), and p.N766N (rs6196) genotype groups. It was not possible to evaluate the association of these parameters

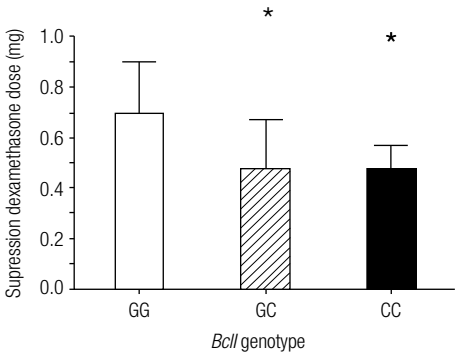

Figure 1. Mean values of dexamethasone dose (mg) that suppressed plasma and salivary cortisol (cutoff levels of plasma cortisol was < 50 nmol/L or 1.8 µg/dL, and salivary cortisol was 2.6 nmol/L or 92 ng/dL) in GG-carriers (white bars), CG-carriers (striped bars), CC-carriers (black bars). Dose was significantly higher in GG-genotype carriers. * $P = 0.03$.

with p.ER22/23EK, p.P750P, and the intron H polymorphisms since they were rarely observed in the Brazilian population.

The GR binding affinity (K_d) and the concentration of DEX (mol/L) that caused 50% inhibition of peripheral mononuclear cell proliferation (IC_{50}) did not differ among genotype groups. In addition, we did not find any differences in BMI between any genotype groups.

DISCUSSION

The heterogeneous ethnic origin of the Brazilian population may contribute both to decrease inter-population diversity and to restore part of total genetic diversity. The *NR3C1* genetic background is well established in several countries of Europe whose immigrants came to Brazil. However, there are few studies on *NR3C1* genetic background of African descendants, and most of them regard African-Americans. It is important to point out that African-Brazilians have different genetic background from African-Americans, which can be demonstrated by studies on several polymorphisms, especially linked to the β^s hemoglobin mutation (24,25). Therefore, the study on *NR3C1* genetic background in all subpopulations that generated the current Brazilian population might provide important anthropological information.

The single nucleotide polymorphism database dbSNP currently lists 2617 SNPs in the human GR gene locus. However, of these polymorphisms, only 64 are in the coding region, and only 39 lead to amino acid changes (http://www.ncbi.nlm.nih.gov/SNP/).

The 363S was detected mainly in heterozygosis in a frequency of 3.4%, only in Caucasian and African-origin subjects. Our data are in accordance to studies in randomly selected European populations, which have reported a frequency of the 363S allele of 3-7% (20,26-29). Interestingly, we observed no 363S allele in Brazilian subjects of Asian origin, similar to a study in South Asian subjects living in the United Kingdom, in which the 363S allele frequency was very low (0.3%) (30). Recently, the p.N363S polymorphism was neither detected in a Japanese population (31) nor in Chinese Han individuals, a subset of Asian-origin population (32).

The 363S polymorphic allele, located at exon 2, which alters the N-terminal transactivation domain, has been associated with increased sensitivity to GCs (20). Studies in Australia have linked the 363S allele with raised body mass index (BMI) in healthy normotensive adults of Anglo-Celtic descent (26), and with coronary artery disease (33). In addition, it was found that the p.N363S polymorphism is associated with central obesity in European men from northeast England (27), and in overweight French Caucasians men with type 2 *diabetes mellitus* (29). In contrast, studies in Swedish and Danish populations have not found an association of N363S with altered sensitivity to GCs or with obesity (28,34).

In our study, among all genotyped subjects, we evaluated the association of p.N363S variant and BMI, early morning serum or salivary cortisol concentrations at baseline and after administration of different doses of DEX, GR binding affinity (K_d), and the concentration of DEX (mol/L) that caused 50% inhibition of peripheral mononuclear cell proliferation in 40 healthy subjects. We found no association between p.N363S variant and each of these parameters. Indeed, given the low prevalence of p.N363S variant in the overall population, mainly in subjects of Asian origin, it is unlikely that the presence of p.N363S variant is the mechanism underlying GC sensitivity, obesity, or other dysmetabolic features.

We detected the p.ER22/23EK polymorphism in a low frequency (2%) and exclusively in Caucasian Brazilian subpopulation. Although our Japanese descendants sample size was limited, the p.ER22/23EK polymorphism was not detected in a population of Chinese Han individuals, another subset of Asian-origin population (32).

Recently it has been elucidated that p.ER22/23EK changes the balance between the A and B translational

isoforms of the GR protein (35). The presence of the p.ER22/23EK polymorphism favors the translation initiation from methionine 1 (GR-A) over initiation from methionine 27 (GR-B), resulting in a decrease in transactivation with normal transrepression (35). This polymorphism has been associated with relative glucocorticoid resistance, and with greater insulin sensitivity and lower total and low-density lipoprotein (LDL) cholesterol levels (22). Furthermore, it was reported that p.ER22/23EK SNP was more prevalent in older individuals, suggesting that it may have a beneficial effect on survival. In 402 men studied in a follow-up of 4 years, while all the p.ER22/23EK carriers survived, 19% of non-carriers died. In addition, the p.ER22/23EK SNP has been associated with a beneficial body composition and muscle strength in young adults (36,37). In the present study, we found no association between p.ER22/23EK polymorphism and BMI, baseline and after different doses of DEX early morning serum or salivary cortisol concentrations, GR binding affinity (K_d), and the DEX IC_{50} of peripheral mononuclear cell proliferation, suggesting no association between this polymorphism and glucocorticoid resistance.

BclI polymorphism has been associated with increased GC sensitivity determined by skin blanching response to topical GCs and increased cortisol suppression after low-dose DEX (19,38,39). In a large group of Dutch elderly individuals, subjects with the G allele had lower cortisol levels after dexamethasone suppression test as well as a tendency towards a decreased lean body mass and abdominal fat distribution, high blood pressure, altered insulin sensitivity, and cardiovascular risk factors, suggesting hypersensitivity to GC (38). In 42 blood donors, association between the *BclI* variant genotype and an increased *in vitro* sensitivity to GCs was observed by DEX-mediated inhibition of concanavalin-A-stimulated PMBC (40). On the contrary, our data show no differences in BMI, Kd, IC_{50}, and early morning serum or salivary cortisol concentrations at baseline and after dexamethasone suppression tests among *BclI* genotype groups. However, the mean values of DEX dose that suppressed plasma and salivary cortisol differed among the three genotype groups. DEX dose was higher in homozygous G-allele carriers, compared with GC heterozygous and also with CC-carriers. Variable absorption and metabolism of dexamethasone may influence the result of the DEX suppression tests, thus, in order to avoid these interferences, dexamethasone levels were simultaneously measured with cortisol to ensure that all individuals have achieved adequate plasma dexamethasone concentrations. Besides, no correlation was observed between DEX suppressive dose and BMI, which suggests that the degree of obesity is not responsible for the differences observed between the genotypes.

Recently, the *BclI* polymorphism have been associated with younger age of onset, unfavorable course, and higher inflammatory activity in girls with idiopatic juvenile arthritis (41). The GG genotype was also associated with more severe lung damage in cystic fibrosis patients (42) and with Crohn's disease (43), suggesting a possible decrease in glucocorticoid sensitivity. Conversely, in Crohn's disease, GG genotype was also related with increased response to glucocorticoid treatment (44). It is possible that the *BclI* polymorphism exerts different influence depending on tissue, disease and ethnicity (44). Since *BclI* is an intronic polymorphism, it is also possible that its effect on GR activity may occur by selectively acting on repressor or enhancer sites (42). Indeed, one of the major limitations of genetic association studies is the lack of reproducibility. Problems with the number of individuals studied, racial heterogeneity, population stratification (founder effect), functionality and multiple testing often mean that studies are not reproducible. Further analysis is required to replicate any of these findings and to understand the mechanism underlying the observed association.

Studies on the relationship between GR variants and cardiovascular disease (CVD) risk using different parameters of atherosclerosis have also yielded conflicting results (33,45,46). A recent study based on follow-up of a large population has examined the association of *BclI*, p.N363S, p.ER22/23EK, and *GR-9*β glucocorticoid receptor variants with changes in cortisol sensitivity and CVD. *BclI*, p.N363S and p.ER22/23K- GR9β glucocorticoid receptor haplotypes were not associated with the risk of myocardial infarction, CVD, high sensitivity C reactive protein and IL-6 levels, and intima-media thickness (47). These findings replicate and further support the results of a previous study performed in a population of 552 elderly persons (45). On the other hand, another study evaluating p.N363S, p.ER22/23EK, *BclI* and *GR-9*β demonstrated that only in men, the *BclI* variant was associated with a 34% higher CVD risk and

the GR9β variant with a 41% higher CVD risk (48). However, the authors suggest that these results should be replicated in other studies.

We also detected, in the Brazilian population, the G>T change, at intron D/exon5 (rs6188). Similar frequency (GG:64.4/GT:25.6/TT:10.0%) was reported in 90 American subjects (databases NCBI-dbSNP). In our study, for the rs6188 polymorphism, the African and Caucasian subpopulations were in Hardy-Weinberg equilibrium, whereas the Asian subpopulation was not. The Asian subpopulation evaluated in the present study showed a small frequency of the T allele. Similarly, a very low frequency of the T allele was reported (databases NCBI-dsSNP) in 91 Japanese subjects (GG:83.7/GT:1.63) and in 90 Han Chinese subjects (GG:87.8/GT:9.8/TT:2.4). It is important to point out that the subjects of our Asian subpopulation are from a Japanese colony, located at Southwest of Sao Paulo State, which present a very high number of consanguineous marriages. The rs6188 polymorphism has been associated neither with altered glucocorticoid sensitivity nor with any other disease.

Furthermore, other genetic variants were found at low frequencies, some of them in a single individual, and were not useful for genetic association studies. However, these rare alleles could be informative, in the future, in familial analysis of inherited diseases associated with *NR3C1*. For the p.D678D polymorphism (rs258751), the African and Caucasian subpopulations were in Hardy-Weinberg equilibrium, whereas the Asian subpopulation was not. Frequency of CC:95.1% and CT:4.9% was reported in 90 American subjects (databases NCBI-dbSNP). As we can observe, there was no TT genotype in the American population. On the other hand, we observed two subjects from Asian-Brazilian subpopulation with TT homozygous genotype; probably this was the cause of the absence of Hardy-Weinberg equilibrium. This polymorphism was also described in homozygosis in Japanese subjects resident in Japan (31). However, differently from the Asian-Brazilian population, in the Japanese population this polymorphism was on Hardy-Weinberg equilibrium. Once again, we can speculate that, due to a greater number of consanguineous marriage in the Asian-descendant Brazilian subpopulation, there was a greater chance of inheriting the T allele. Probably, more generations would be necessary to obtain the Hardy-Weinberg equilibrium. In addition, in exon 9, we observed the p.N766N (rs6196) polymorphism and two rare GR variants: a heterozygous p.P750P (rs67300719) polymorphism and a C>G substitution, located in the 9 base pairs before the end of intron H (rs72542757). The African, Caucasian, and Asian subpopulations were in Hardy-Weinberg equilibrium for rs6196. It is interesting to point out that the rs72542757 and rs67300719 variant alleles were found only in the Asian-Brazilian subpopulation in an allelic frequency of 1%. Indeed, Koyano and cols. studying 265 subjects in Japan found these allelic variations in a frequency of 1.5%, suggesting that some rare polymorphisms are more frequently associated with certain ethnic groups (31).

In conclusion, this is the first study evaluating *NR3C1* polymorphisms in Brazilians descendents from Caucasian, African and Asian populations, reproducing the heterogeneous ethnic background of the Brazilian population. In this conservative gene, the genotypic frequencies observed in each subpopulation are similar to worldwide ancestry populations. In addition, the *BclI* polymorphism was associated with altered GC sensitivity in the HPA axis, but not in PBMC, suggesting a tissue-specific influence of *BclI* SNP.

Acknowledgements: the authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) for the Financial Support: grant nº 03/00327-8 and grant no. 05/57033-1.

Disclosure: no potential conflict of interest relevant to this article was reported.

REFERENCES

1. Evans RM. The steroid and thyroid hormone receptor superfamily. Science. 1988;240(4854):889-95.
2. Bamberger CM, Schulte HM, Chrousos GP. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev. 1996;17(3):245-61.
3. Francke U, Foellmer BE. The glucocorticoid receptor gene is in 5q31-q32 [corrected]. Genomics. 1989;4(4):610-2.
4. Encio IJ, Detera-Wadleigh SD. The genomic structure of the human glucocorticoid receptor. J Biol Chem. 1991;266(11):7182-8.
5. Zhou J, Cidlowski JA. The human glucocorticoid receptor: one gene, multiple proteins and diverse responses. Steroids. 2005;70(5-7):407-17.
6. de Castro M, Elliot S, Kino T, Bamberger C, Karl M, Webster E, et al. The non-ligand binding beta-isoform of the human glucocorticoid receptor (hGR beta): tissue levels, mechanism of action, and potential physiologic role. Mol Med. 1996;2(5):597-607.
7. Yudt MR, Cidlowski JA. The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene. Mol Endocrinol. 2002;16(8):1719-26.
8. Kino T, Manoli I, Kelkar S, Wang Y, Su YA, Chrousos GP. Glucocorticoid receptor (GR) beta has intrinsic, GRalpha-independent transcriptional activity. Biochem Biophys Res Commun. 2009;381(4):671-5.

9. Colli LM, do Amaral FC, Torres N, de Castro M. Interindividual glucocorticoid sensitivity in young healthy subjects: the role of glucocorticoid receptor alpha and beta isoforms ratio. Horm Metab Res. 2007;39(6):425-9.
10. Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Mol Cell Endocrinol. 2009;300(1-2):7-16.
11. Charmandari E, Kino T. Chrousos syndrome: a seminal report, a phylogenetic enigma and the clinical implications of glucocorticoid signalling changes. Eur J Clin Invest. 2010;40(10):932-42.
12. De Antonio SR, Blotta HM, Mamoni RL, Louzada P, Bertolo MB, Foss NT, et al. Effects of dexamethasone on lymphocyte proliferation and cytokine production in rheumatoid arthritis. J Rheumatol. 2002;29(1):46-51.
13. Chriguer RS, Roselino AM, de Castro M. Glucocorticoid sensitivity and proinflammatory cytokines pattern in pemphigus. J Clin Immunol. 2012;32(4):786-93.
14. Carlotti AP, Franco PB, Elias LL, Facincani I, Costa EL, Foss N, et al. Glucocorticoid receptors, in vitro steroid sensitivity, and cytokine secretion in idiopathic nephrotic syndrome. Kidney Int. 2004;65(2):403-8.
15. De Antonio SR, Saber LT, Chriguer RS, de Castro M. Glucocorticoid resistance in dialysis patients may impair the kidney allograft outcome. Nephrol Dial Transplant. 2008;23(4):1422-8.
16. Leung DY, de Castro M, Szefler SJ, Chrousos GP. Mechanisms of glucocorticoid-resistant asthma. Ann N Y Acad Sci. 1998;840:735-46.
17. Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE, et al. Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. J Clin Endocrinol Metab. 1998;83(1):47-54.
18. Chriguer RS, Elias LL, da Silva IM Jr, Vieira JG, Moreira AC, de Castro M. Glucocorticoid sensitivity in young healthy individuals: in vitro and in vivo studies. J Clin Endocrinol Metab. 2005;90(11):5978-84.
19. Panarelli M, Holloway CD, Fraser R, Connell JM, Ingram MC, Anderson NH, et al. Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J Clin Endocrinol Metab. 1998;83(6):1846-52.
20. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab. 1998;83(1):144-51.
21. Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, et al. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. J Rheumatol. 2001;28(11):2383-8.
22. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, et al. A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes. 2002;51(10):3128-34.
23. Karl M, Lamberts SW, Detera-Wadleigh SD, Encio IJ, Stratakis CA, Hurley DM, et al. Familial glucocorticoid resistance caused by a splice site deletion in the human glucocorticoid receptor gene. J Clin Endocrinol Metab. 1993;76(3):683-9.
24. Bortolini MC, Zago MA, Salzano FM, Silva-Junior WA, Bonatto SL, da Silva MC, et al. Evolutionary and anthropological implications of mitochondrial DNA variation in African Brazilian populations. Hum Biol. 1997;69(2):141-59.
25. Figueiredo MS, Kerbauy J, Goncalves MS, Arruda VR, Saad ST, Sonati MF, et al. Effect of alpha-thalassemia and beta-globin gene cluster haplotypes on the hematological and clinical features of sickle-cell anemia in Brazil. Am J Hematol. 1996;53(2):72-6.
26. Lin RC, Wang WY, Morris BJ. High penetrance, overweight, and glucocorticoid receptor variant: case-control study. BMJ. 1999;319(7221):1337-8.
27. Dobson MG, Redfern CP, Unwin N, Weaver JU. The N363S polymorphism of the glucocorticoid receptor: potential contribution to central obesity in men and lack of association with other risk factors for coronary heart disease and diabetes mellitus. J Clin Endocrinol Metab. 2001;86(5):2270-4.
28. Rosmond R, Bouchard C, Bjorntorp P. Tsp509I polymorphism in exon 2 of the glucocorticoid receptor gene in relation to obesity and cortisol secretion: cohort study. BMJ. 2001;322(7287):652-3.
29. Roussel R, Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G. The N363S polymorphism in the glucocorticoid receptor gene is associated with overweight in subjects with type 2 diabetes mellitus. Clin Endocrinol (Oxf). 2003;59(2):237-41.
30. Syed AA, Irving JA, Redfern CP, Hall AG, Unwin NC, White M, et al. Low prevalence of the N363S polymorphism of the glucocorticoid receptor in South Asians living in the United Kingdom. J Clin Endocrinol Metab. 2004;89(1):232-5.
31. Koyano S, Saito Y, Sai K, Kurose K, Ozawa S, Nakajima T, et al. Novel genetic polymorphisms in the NR3C1 (glucocorticoid receptor) gene in a Japanese population. Drug Metab Pharmacokinet. 2005;20(1):79-84.
32. Duan ZX, Gu W, Du DY, Hu P, Jiang DP, Zhu PF, et al. Distributions of glucocorticoid receptor gene polymorphisms in a Chinese Han population and associations with outcome after major trauma. Injury. 2009;40(5):479-83.
33. Lin RC, Wang XL, Morris BJ. Association of coronary artery disease with glucocorticoid receptor N363S variant. Hypertension. 2003;41(3):404-7.
34. Echwald SM, Sorensen TI, Andersen T, Pedersen O. The Asn363Ser variant of the glucocorticoid receptor gene is not associated with obesity or weight gain in Danish men. Int J Obes Relat Metab Disord. 2001;25(10):1563-5.
35. Russcher H, van Rossum EF, de Jong FH, Brinkmann AO, Lamberts SW, Koper JW. Increased expression of the glucocorticoid receptor-A translational isoform as a result of the ER22/23EK polymorphism. Mol Endocrinol. 2005;19(7):1687-96.
36. van Rossum EF, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, Kemper HC, et al. The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. J Clin Endocrinol Metab. 2004;89(8):4004-9.
37. van Rossum EF, Feelders RA, van den Beld AW, Uitterlinden AG, Janssen JA, Ester W, et al. Association of the ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in elderly men. Am J Med. 2004;117(3):158-62.
38. van Rossum EF, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W, et al. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clin Endocrinol (Oxf). 2003;59(5):585-92.
39. Weaver JU, Hitman GA, Kopelman PG. An association between a Bc1I restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. J Mol Endocrinol. 1992;9(3):295-300.
40. Cuzzoni E, De Iudicibus S, Bartoli F, Ventura A, Decorti G. Association between BclI polymorphism in the NR3C1 gene and in vitro individual variations in lymphocyte responses to methylprednisolone. Br J Clin Pharmacol. 2012;73(4):651-5.
41. Kostik MM, Klyushina AA, Moskalenko MV, Scheplyagina LA, Larionova VI. Glucocorticoid receptor gene polymorphism

and juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2011;9(1):2.

42. Corvol H, Nathan N, Charlier C, Chadelat K, Le Rouzic P, Tabary O, et al. Glucocorticoid receptor gene polymorphisms associated with progression of lung disease in young patients with cystic fibrosis. Respir Res. 2007;8:88.
43. Decorti G, De Iudicibus S, Stocco G, Martelossi S, Drigo I, Bartoli F, et al. Glucocorticoid receptor polymorphisms in inflammatory bowel disease. Gut. 2006;55(7):1053-4.
44. De Iudicibus S, Stocco G, Martelossi S, Drigo I, Norbedo S, Lionetti P, et al. Association of BclI polymorphism of the glucocorticoid receptor gene locus with response to glucocorticoids in inflammatory bowel disease. Gut. 2007;56(9):1319-20.
45. Kuningas M, Mooijaart SP, Slagboom PE, Westendorp RG, van Heemst D. Genetic variants in the glucocorticoid receptor gene (NR3C1) and cardiovascular disease risk. The Leiden 85-plus Study. Biogerontology. 2006;7(4):231-8.
46. Goracy I, Goracy J, Safranow K, Skonieczna-Zydecka K, Ciechanowicz A. Association of glucocorticoid receptor gene NR3C1 genetic variants with angiographically documented coronary artery disease and its risk factors. Arch Med Res. 2013;44(1):27-33.
47. van den Akker EL, Koper JW, van Rossum EF, Dekker MJ, Russcher H, de Jong FH, et al. Glucocorticoid receptor gene and risk of cardiovascular disease. Arch Intern Med. 2008;168(1):33-9.
48. Koeijvoets KC, van der Net JB, van Rossum EF, Steyerberg EW, Defesche JC, Kastelein JJ, et al. Two common haplotypes of the glucocorticoid receptor gene are associated with increased susceptibility to cardiovascular disease in men with familial hypercholesterolemia. J Clin Endocrinol Metab. 2008;93(12):4902-8.