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

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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 (Kd), and DEX-50% inhibition (IC50) 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/GC:41.1/GG:0.8), 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:72/TC:20/CC:2.4), rs67300719 (n = 137; CC:99.3/CT:0.7), and rs72542757 (n = 137; CC:99.3/GG: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 Kd, IC50, 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

RESUMO

Objetivo: Este estudo avalia polimorfismos (SNPs) do NR3C1 na população brasileira, que possui origem étnica heterogênea. Materiais e métodos: Os SNPs do NR3C1 foram avaliados em brasileiros de ancestralidade de 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 (Kd) e inibição por DEX de 50% da proliferação de mononucleares estimulada por concanavalina-A (IC50). Discriminação alélica de p.N363S (rs6195), p.ER22/23EK (rs6189-6190) e BclI (rs41423247) foi realizada por PCR em tempo real. Exons 3 a 9 e transições exôn/intrôn 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/GC:41.1/GG:0.8), 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:72/TC:20/CC:2.4), rs67300719 (n = 137; CC:99.3/CT:0.7), e rs72542757 (n = 137; CC:99.3/GG: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. Kd, IC50, 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

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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, ubiquitination, 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 (IC50) 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 [3H]-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/m².
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 descendants. 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 (ANOVA) 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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BclI</strong></td>
<td>FWD: 5’-CAGGGTTCTGCTATAAGTAGACA-3’</td>
</tr>
<tr>
<td>REW: 5’-GACCATAGTTGGCAACACATTCC-3’</td>
<td>VT: 5’-CTCTAAAGAGATTGATGACG-3’</td>
</tr>
<tr>
<td><strong>N363S</strong></td>
<td>FWD: 5’-GTCACTCCACCACTCCCGTG-3’</td>
</tr>
<tr>
<td>REW: 5’-GCAATGTGCATCTCAGCTCCT-3’</td>
<td>VT: 5’-ACCTATTCCATTTCGG-3’</td>
</tr>
<tr>
<td><strong>ER22/23EK</strong></td>
<td>FWD: 5’-AGAGAAAAACCCACGAGCTGT-3’</td>
</tr>
<tr>
<td>REW: 5’-CAGTAGCTCCTCTTCTTAGGGTTTA-3’</td>
<td>VT: 5’-CACATCTCCCTTCTCTGA-3’</td>
</tr>
</tbody>
</table>

REW: reverse; FWD: forward; WT: wild type; VT: variant.
NR3C1 genotypes and glucocorticoid sensitivity

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:29.6/TT:2.3 (n = 150), 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:80.2/TC:17.4/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 heterozygous p.P750P polymorphism (rs67300719) (n = 137; CC:99.3/CG:0.7); and 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 p.(N766N) polymorphism (rs6668) in a frequency of CC:99.6/GA:0.4/AG:0.0 (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 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 NR3CI 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).

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

<table>
<thead>
<tr>
<th>Location</th>
<th>db SNP Accession</th>
<th>Minor change</th>
<th>Rare allele</th>
<th>Caucasian (Genotypic frequency)</th>
<th>African (Genotypic frequency)</th>
<th>Asian (Genotypic frequency)</th>
<th>Total (Genotypic frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>rs 6189/6190 (p.E2223EK)</td>
<td>G/A</td>
<td>A</td>
<td>230 (GG:99.5/AG:0.5)</td>
<td>31 (GG:100)</td>
<td>3 (GG:100)</td>
<td>264 (GG:99.6/GA:0.4)</td>
</tr>
<tr>
<td>Exon 8</td>
<td>rs 258751 (p.D678D)</td>
<td>C/T</td>
<td>T</td>
<td>50 (CC:98/CT:1/T:2)</td>
<td>50 (CC:98/CT:1/T:2)</td>
<td>50 (CC:98/CT:1/T:2)</td>
<td>150 (CC:98/CT:1/T:2)</td>
</tr>
<tr>
<td>Intron H</td>
<td>rs72542757 (IVS8 -9)</td>
<td>C/G</td>
<td>G</td>
<td>48 (CC:100)</td>
<td>40 (CC:100)</td>
<td>49 (CC:98/CG:2)</td>
<td>137 (CC:99.3/CG:0.7)</td>
</tr>
<tr>
<td>Exon 9</td>
<td>rs67300719 (p.P750P)</td>
<td>C/T</td>
<td>T</td>
<td>48 (CC:100)</td>
<td>40 (CC:100)</td>
<td>49 (CC:98/CG:2)</td>
<td>137 (CC:99.3/CT:0.7)</td>
</tr>
</tbody>
</table>

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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.

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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
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, another 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 (Kd), 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 (Kd), and the DEX IC50 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, IC50, 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 idiopathic 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-9β glucocorticoid receptor variants with changes in cortisol sensitivity and CVD. BclI, p.N363S and p.ER22/23EK- 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-9β 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-dbSNP) 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 NR3CI. 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. This polymorphism was associated with certain ethnic groups (31).

In conclusion, this is the first study evaluating NR3CI polymorphisms in Brazilians descendants 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.

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NR3C1 genotypes and glucocorticoid sensitivity


