Endocrine (2014) 47:740–748
DOI 10.1007/s12020-014-0324-9

ENDOCRINE GENETICS/EPIGENETICS

Polymorphisms in NR3C1 gene associated with risk of metabolic syndrome in a Chinese population

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Received: 11 February 2014 / Accepted: 2 June 2014 / Published online: 8 August 2014
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Abstract Polymorphisms of the NR3C1 (glucocorticoid receptor) gene have been reported to be associated with altered glucocorticoids sensitivity and changes in body composition and metabolic parameters. This study explored the relationship between single nucleotide polymorphisms (SNPs) of the NR3C1 gene and metabolic syndrome (MetS) in a Chinese population. Fourteen tag-SNPs and five functionally important SNPs in the NR3C1 gene were genotyped in MetS patients ($n = 431$) and normal controls ($n = 461$) using the high-throughput Sequenom genotyping platform. Genotype, allelic and haplotype associations were examined using logistic regression and Haploview. There are four SNPs significantly associated with MetS. The T allele of rs2963156 was associated with an increased risk effect for MetS (adjusted OR = 1.66, 95 % CI 1.25–2.22, $P = 0.001$). By contrast, rs10052957 A allele carriers were significantly associated with a decreased risk of MetS (adjusted OR = 0.58, 95 % CI 0.42–0.80, $P = 0.001$). Rs41423247 GG genotype (adjusted OR = 2.01, 95 % CI 1.25–3.22, $P = 0.004$), and rs7701443 AA genotype (adjusted OR = 1.88, 95 % CI 1.24–2.83, $P = 0.003$) were significantly associated with an increased risk of MetS. Haplotype CGGA is risk conferring (adjusted OR = 1.53, 95 % CI 1.06–2.20, $P = 0.023$), whereas haplotype CCAG was protective (adjusted OR = 0.30, 95 % CI 0.20–0.47, $P < 0.001$). Polymorphism of NR3C1 gene is associated with MetS and may contribute to the susceptibility of MetS.

Keywords Glucocorticoid receptor · NR3C1 · Polymorphism · Metabolic syndrome · Risk factor

Introduction

Metabolic syndrome (MetS) is a common, multicomponent condition characterized by insulin resistance, dyslipidemia, abdominal obesity, and hypertension that is associated with an increased risk of type 2 diabetes mellitus, cardiovascular diseases, and atherosclerosis [1]. MetS has become prevalent in Western and Asian countries due to both environmental factors and lifestyle changes. Environmental influences such as chronic stress, behavioral and metabolic disturbances, dietary deficiency, and infection have now emerged as contributors to the development of metabolic disease. Epidemiological data suggest strong association between chronic stress exposure and metabolic disease. The data indicate that glucocorticoids (GCs) changes under chronic social stress may influence the process involved in food intake and body weight regulation, leading to metabolic disorders over long-term, and repeated exposures to stress [2]. At the cellular level, the action of GCs is mediated by an intracellular protein, the glucocorticoid receptor

Electronic supplementary material The online version of this article (doi:10.1007/s12020-014-0324-9) contains supplementary material, which is available to authorized users.

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(GR), which is coded by gene NR3C1 [3–5]. Inter-individual variations in tissue sensitivity to GCs have been described within the normal population and have been partly attributed to polymorphisms in NR3C1 gene [6]. The NR3C1 gene is located on chromosome 5p31–32, spanning approximately 121 kb in length with 9 exons and 8 introns. Several polymorphisms of the NR3C1 gene have been reported to be associated with altered GCs sensitivity and changes in body composition and metabolic parameters [7–9].

The N363S polymorphism of the NR3C1 gene is associated with increased sensitivity to GCs, a trend toward increased body mass index (BMI) [10]. The G allele of *Bcl*I polymorphism is also associated with increased sensitivity to GCs, which may contribute to increased abdominal obesity [11]. A third polymorphism of ER22/23EK is associated with a relative resistance to GCs [12]. Associations with GCs resistance and healthier metabolic profile observed in the *Tth111*I carriers are likely to arise as a result of the ER22/23EK polymorphism [13]. Other polymorphisms including D401H and A3669G in the NR3C1 gene associated with metabolic profile are deemed that they may contribute to alterations in tissue sensitivity to GCs [14, 15].

However, the relationship between NR3C1 variation and metabolic parameters are inconsistent in different population because the general frequency of polymorphisms varies greatly between ethnic populations [9, 16]. In previous cross-sectional study, we found that polymorphisms of the NR3C1 gene (rs2963156, rs41423247, rs7701443, rs17209251, and rs10052957) are associated with cardiovascular risk factors in a healthy Chinese Han population [17]. In order to determine the association between polymorphisms of NR3C1 gene and MetS in Chinese Han population and to explore the possible linkage between NR3C1 gene polymorphism and cardiovascular diseases, an association study in MetS cases and controls was conducted.

Materials and methods

Subjects

We recruited 431 MetS patients and 461 non-MetS controls from staffs in 8 companies who took regular physical examination from January to September 2012 at the physical examination center of Xuanwu Hospital, Capital Medical University. All subjects were genetically unrelated ethnic Han Chinese and were resided in Beijing. The cases are required to be aged from 30 to 65 years old. Cases and controls were frequency-matched according to age (±5 years), gender, and working company. To assure comparability of the findings, all participants were examined by the physicians who were specially trained for the study. Both the hospital and university research ethical committees approved the study, and written informed consents were obtained from all participants.

Each participant was interviewed and completed a structured questionnaire to collect information on demographic variables, health-related behaviors, and medical and family histories. Current cigarette smoking was defined as ≥1 filter per day. Alcohol use was defined as ≥1 times intake of wine/beer/cider/spirits per week. Physical activity levels were defined as walking or riding ≥ 15 min/day or doing sports or physical exercise >2 h/week.

Definition of MetS

Definition of MetS was based on clinical criteria from a previous joint interim statement of the International Diabetes Federation (IDF); American Heart Association (AHA); National Heart, Lung and Blood Institute (NHLBI); World Heart Federation; International Atherosclerosis Society and International Association for the Study of Obesity [18]. In summary, the presence of any 3 of 5 risk factors constitutes a diagnosis of MetS: (1) elevated waist circumference (WC): ≥90 cm in males, ≥80 cm in females; (2) elevated triglycerides (TG): ≥150 mg/dL (1.7 mmol/L) in both genders (whereas drug treatment for elevated triglycerides is an alternate indicator); (3) reduced high-density lipoprotein cholesterol (HDL-C): <40 mg/dL (1.0 mmol/L) in males, <50 mg/dL (1.3 mmol/L) in females (whereas drug treatment for reduced HDL-C is an alternate indicator); (4) elevated blood pressure: systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg in both genders (whereas antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator); (5) Elevated fasting glucose (FPG): ≥100 mg/dL (5.6 mmol/L) in both gender (whereas drug treatment of elevated glucose is an alternate indicator).

Measurement of anthropometric parameters

Weight, height, and WC were measured and BMI was calculated. Waist diameters were measured to the nearest 1.0 cm at the height of the navel upon breath intake using a non-extendable linen measure. Blood pressure was measured on the right arm using a mercury sphygmomanometer after at least 5 min of rest in the sitting position. Measurements were made three times and the average value was used in the analyses. Following an overnight fast, venous blood samples were collected and promptly centrifuged for laboratory measurements and genotype analysis. Total cholesterol (TC), HDL-C and triglycerides

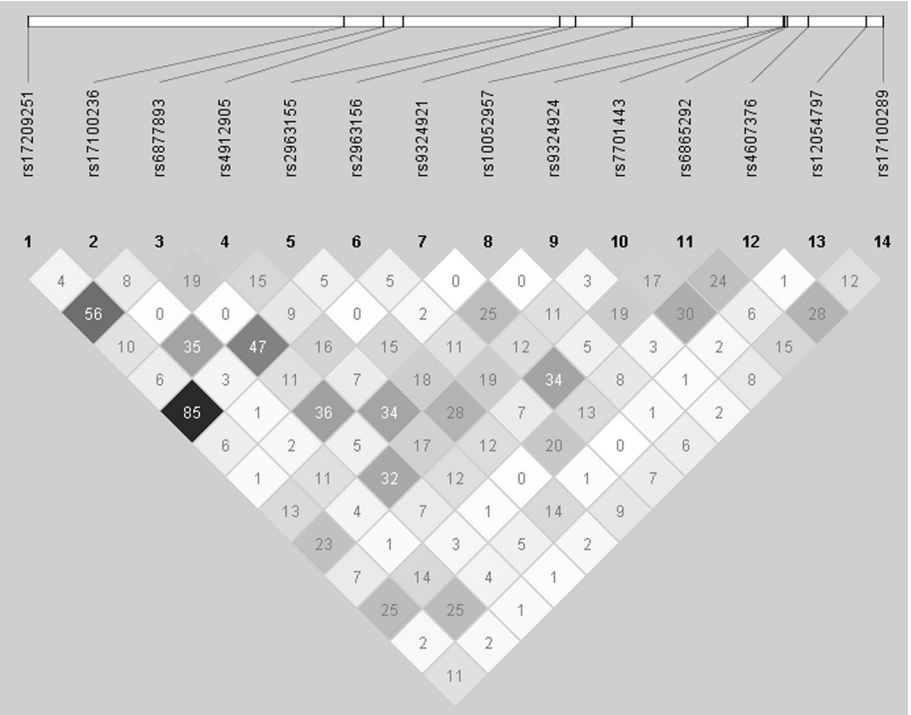

Fig. 1 Linkage disequilibrium ($r^2 \times 100$) between SNPs in the NR3C1 gene

(TG) were measured using standard laboratory methods (Hitachi autoanalyzer 7060, Japan); low density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald method. Fasting plasma glucose (FPG) levels were measured by the glucose oxidase method.

Selection of SNPs and genotyping

Fourteen tagging single nucleotide polymorphisms (tag-SNPs) across the NR3C1 gene were identified using the methods described by Yan et al. [17]. A linkage disequilibrium (LD) threshold of $r^2 \geq 0.8$ and minor allele frequency (MAF) ≥ 0.1 was used to select the tag-SNPs (Fig. 1). In addition to the 14 tag-SNPs, other five SNPs including rs56149945 (N363S), rs41423247 (*Bcl*I), rs6189/6190 (ER22/23EK), rs6198 (A3669G), and D401H were added to the set of markers based on reported evidence of their functional/clinical significance [10–16]. These polymorphisms have been shown associated with metabolic parameters and body composition.

Genomic DNA was extracted from peripheral white blood cells of participants using a DNeasy tissue kit (Qiagen) according to the manufacturer's instructions. The 19 SNPs were genotyped using the high-throughput Sequenom matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) iPLEX platform. Laboratory personnel and the readers of genotyping were blinded to the status of cases and controls. Genotyping was repeated in 5 % of samples for verification and quality control. Quality control testing revealed that genotype data had an error rate <0.1 %.

Statistical analyses

Clinical parameters were compared between the MetS cases and MetS-free controls using Student's *t* test. Each genetic marker was tested for Hardy–Weinberg equilibrium using χ^2 test among controls. Univariate and multivariate logistic regression analysis were performed to estimate crude and adjusted odds ratios (ORs) and 95 % confidence intervals (95 % CIs) as a measure of association of genotypes with the risk of MetS, as well as corresponding *P* values. Various models of inheritance including additive, dominant, and recessive were fit. All statistics analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The association between the risk haplotype and MetS was assessed by the Chi-square test using Haploview software (http://www.broad.mit.edu/haploview/haploview).

Results

Basic characteristics of the study subjects

Table 1 shows the demographic and clinical characteristics of MetS and non-MetS controls. The frequency of smoking and physical inactivity was greater in patients with MetS

Table 1 Characteristics of 892 subjects

Variable	MetS ($n = 431$)	non-MetS ($n = 461$)	P
Age (year)	47.64 ± 8.23	46.63 ± 8.10	0.065
Gender (male/female)	215/246	210/221	0.533
WC (cm)	87.08 ± 7.07	76.49 ± 7.46	<0.001
BMI	26.62 ± 2.32	22.55 ± 2.49	<0.001
SBP (mmHg)	133.66 ± 11.50	118.01 ± 12.75	<0.001
DBP (mmHg)	87.30 ± 9.01	75.22 ± 9.25	<0.001
FPG (mmol/L)	6.53 ± 1.49	5.18 ± 0.69	<0.001
TC (mmol/L)	5.13 ± 0.91	4.70 ± 0.86	<0.001
TG (mmol/L)	2.52 ± 1.98	1.18 ± 0.38	<0.001
HDLC (mmol/L)	1.22 ± 0.33	1.57 ± 0.31	<0.001
LDLC (mmol/L)	2.82 ± 0.85	2.49 ± 0.67	<0.001
Smoking (n, %)	91, 21.11	72, 15.62	0.034
Alcohol use (n, %)	93, 21.58	88, 19.09	0.198
Physical activity (n, %)	309, 71.69	362, 78.52	0.018

Table 2 Characteristics of the studied Tag-SNPs in the NR3C1 gene

SNP ID	Genomic location	Genic position	Alleles (major/minor)	MAF	HWE P
rs17100236	142700919	intron_2	T/C	0.155	0.353
rs6877893	142707386	intron_2	A/G	0.223	0.117
rs2963155	142736197	intron_2	A/G	0.189	0.246
rs2963156	142738689	intron_2	C/T	0.154	0.335
rs9324921	142747933	intron_2	C/A	0.177	0.362
rs41423247	142758768	intron_2	C/G	0.253	0.353
rs10052957	142766894	intron_1	G/A	0.113	0.100
rs9324924	142772677	intron_1	G/T	0.364	0.519
rs7701443	142772843	intron_1	G/A	0.314	0.257
rs6865292	142773183	intron_1	T/C	0.189	0.263
rs4607376	142776725	intron_1	G/A	0.356	0.434
rs12054797	142786095	intron_1	C/T	0.094	0.626

than in controls. WC, BMI, SBP, DBP, serum levels of TC, TG and LDL-C, and the FG level were higher in subjects with MetS, whereas serum levels of HDL-C were found to be significantly lower in subjects with MetS than in subjects without MetS (Table 1).

Identification of polymorphisms in the NR3C1 gene

We were able to successfully genotyped all the 19 SNPs. The overall genotyping rate in markers was high (99.6 %). No variation of N363S, ER22/23EK, A3669G, and D401H were detected in the sample. Of the rest of the 15 SNPs, rs17100289, rs4912905, and rs17209251 were not in Hardy–Weinberg equilibrium in the control group ($P < 0.05$) and hence were excluded. The final analysis was thus based on 12 SNPs (Table 2). Multiple test corrected threshold of α was 0.004, assuming family-wise α level of 0.05 and number of markers ($n = 12$).

Association of NR3C1 gene polymorphisms with MetS

Single SNP analysis of the 12 markers revealed that, four markers (rs2963156, rs41423247, rs10052957, and rs7701443) were associated with MetS at the genotypic/allelic level ($P < 0.004$) (Table 3). After correction for possible confounding variables, these SNPs remained significantly associated with MetS.

Multivariate logistic regression (adjusted for age, gender, smoking, alcohol use, and physical activity) showed that when compared with the homozygous CC genotype, the variant CT genotype and the combined CT/TT genotype of rs2963156 were all associated with an increased risk of MetS (adjusted OR = 1.62, 95 % CI 1.19–2.19, $P = 0.002$; adjusted OR = 1.66, 95 % CI 1.25–2.22, $P = 0.001$) (Table 3). These results indicate that the variant T allele of rs2963156 was the risk allele of MetS. By contrast, rs10052957 A allele carriers were significantly associated with a decreased risk of MetS (adjusted OR = 0.58, 95 % CI 0.42–0.80, $P = 0.001$) (Table 3). Compared with the homozygous CC genotype, rs41423247 GG genotype carriers were significantly associated with an increased risk of MetS (adjusted OR = 2.01, 95 % CI 1.25–3.22, $P = 0.004$). Similarly, AA genotype of rs7701443 compared with the GG genotype was associated with a significant risk effect for MetS (adjusted OR = 1.88, 95 % CI 1.24–2.83, $P = 0.003$) (Table 3).

Association of NR3C1 gene polymorphisms with components of MetS

Multivariate logistic regression analysis (adjusted for age, gender, smoking, alcohol use, and physical activity) was then performed to test for genotype/allele associations between each of the four significant associated markers and metabolic phenotypes. For elevated WC, significant allelic associations for rs2963156 and rs7701443, and genotype associations for rs41423247 and rs7701443 were observed (Table 4). The AA genotype of rs7701443 was associated with reduced HDL-C ($P = 0.004$). For elevated BP, rs2963156 and rs41423247 showed significant allelic associations (Table 4). The G allele of rs10052957 was associated with low FPG ($P < 0.001$).

Table 3 Association of NR3C1 gene type/alleles and MetS

Variable	Case no. (%)	Control no. (%)	Crude OR (95 % CI)	*P* value	Adjusted OR (95 % CI)[a]	*P* value
rs17100236						
TT	311 (72.2)	307 (66.6)	1	–	1	–
TC	111 (25.8)	142 (30.8)	0.77 (0.58, 1.04)	0.084	0.76 (0.57, 1.03)	0.072
CC	9 (2.1)	12 (2.6)	0.74 (0.31, 1.78)	0.502	0.74 (0.31, 1.78)	0.499
TC/CC[b]	120 (27.9)	154 (33.4)	0.77 (0.58, 1.02)	0.072	0.76 (0.57, 1.01)	0.062
TT/TC[c]	422 (97.9)	449 (97.4)	1.25 (0.52, 3.00)	0.613	1.26 (0.52, 3.02)	0.612
rs6877893						
AA	236 (54.8)	269 (58.4)	1	–	1	–
AG	167 (38.7)	174 (37.7)	1.09 (0.83, 1.44)	0.522	1.10 (0.83, 1.45)	0.501
GG	28 (6.5)	18 (3.9)	1.77 (0.96, 3.29)	0.069	1.92 (1.03, 3.59)	0.040
AG/GG[b]	195 (45.2)	192 (41.6)	1.16 (0.89, 1.51)	0.279	1.17 (1.09, 1.85)	0.242
AA/AG[c]	403 (93.5)	443 (96.1)	0.59 (0.32, 1.07)	0.083	0.54 (0.29, 1.00)	0.050
rs2963155						
AA	273 (63.3)	290 (62.9)	1	–	1	–
AG	137 (31.8)	146 (31.7)	1.00 (0.75, 1.33)	0.982	0.98 (0.74, 1.31)	0.913
GG	21 (4.9)	25 (5.4)	0.89 (0.49, 1.63)	0.711	0.87 (0.47, 1.60)	0.651
AG/GG[b]	158 (36.7)	171 (37.1)	0.98 (0.75, 1.29)	0.893	0.97 (0.74, 1.27)	0.812
AA/AG[c]	410 (95.1)	436 (94.6)	1.12 (0.62, 2.03)	0.710	1.14 (0.63, 2.09)	0.660
rs2963156						
CC	277 (64.3)	345 (74.8)	1	–	1	–
CT	133 (30.9)	103 (22.3)	**1.61 (1.19, 2.18)**	**0.002**	**1.62 (1.19, 2.19)**	**0.002**
TT	21 (4.9)	13 (2.8)	2.10 (0.99, 4.09)	0.053	2.02 (0.99, 4.12)	0.052
CT/TT[b]	154 (35.8)	116 (25.1)	**1.65 (1.24, 2.21)**	**0.001**	**1.66 (1.25, 2.22)**	**0.001**
CC/CT[c]	410 (95.1)	448 (97.2)	0.57 (0.28, 1.15)	0.114	0.56 (0.28, 1.14)	0.112
rs9324921						
CC	291 (67.5)	293 (63.6)	1	–	1	–
CA	124 (28.8)	145 (31.5)	0.86 (0.65, 1.15)	0.311	0.88 (0.66, 1.17)	0.378
AA	16 (3.7)	23 (5.0)	0.70 (0.36, 1.35)	0.289	0.71 (0.37, 1.37)	0.302
CA/AA[b]	140 (32.5)	168 (36.5)	0.84 (0.64, 1.11)	0.214	0.85 (0.65, 1.13)	0.266
CC/CA[c]	415 (96.3)	438 (95.0)	1.36 (0.71, 2.61)	0.353	1.36 (0.71, 2.61)	0.359
rs41423247						
CC	203 (47.1)	259 (56.2)	1	–	1	–
CG	176 (40.8)	168 (36.4)	1.34 (1.01, 1.77)	0.042	1.30 (0.98, 1.73)	0.065
GG	52 (12.1)	34 (7.4)	**1.95 (1.22, 3.12)**	**0.005**	**2.01 (1.25, 3.22)**	**0.004**
CG/GG[b]	228 (52.9)	202 (43.8)	1.42 (1.11, 1.88)	0.007	1.42 (1.09, 1.85)	0.009
CC/CG[c]	379 (87.9)	427 (92.6)	0.58 (0.37, 0.91)	0.019	0.56 (0.35, 0.88)	0.012
rs10052957						
GG	356 (82.6)	337 (73.1)	1	–	1	–
GA	74 (17.2)	109 (23.6)	0.64 (0.46, 0.89)	0.009	0.65 (0.46, 0.90)	0.010
AA	1 (0.2)	15 (3.3)	0.06 (0.01, 0.48)	0.008	0.07 (0.01, 0.51)	0.009
GA/AA[b]	75 (17.4)	124 (26.9)	**0.57 (0.41, 0.79)**	**0.001**	**0.58 (0.42, 0.80)**	**0.001**
GG/GA[c]	430 (99.8)	446 (96.7)	14.46 (1.90, 109.96)	0.010	13.63(1.79,103.84)	0.012
rs9324924						
GG	147 (34.1)	154 (33.4)	1	–	1	–
GT	212 (49.2)	219 (47.5)	1.01 (0.76, 1.36)	0.926	1.01 (0.75, 1.35)	0.975
TT	72 (16.7)	88 (19.1)	0.86 (0.58, 1.26)	0.432	0.83 (0.56, 1.22)	0.474
GT/TT[b]	284 (65.9)	307 (66.6)	0.97 (0.73, 1.28)	0.825	0.96 (0.72, 1.26)	0.745
GG/GT[c]	359 (83.3)	373 (80.9)	1.18 (0.83, 1.66)	0.354	1.21 (0.85, 1.71)	0.286

Table 3 continued

Variable	Case no. (%)	Control no. (%)	Crude OR (95 % CI)	*P* value	Adjusted OR (95 % CI)[a]	*P* value
rs7701443						
GG	177 (41.1)	195 (42.3)	1	–	1	–
GA	172 (39.9)	218 (47.3)	0.87 (0.65, 1.16)	0.336	0.83 (0.62, 1.11)	0.237
AA	82 (19.0)	48 (10.4)	**1.88 (1.25, 2.84)**	**0.003**	**1.88 (1.24, 2.83)**	**0.003**
GA/AA[b]	254 (58.9)	266 (57.7)	1.05 (0.81, 1.37)	0.709	1.02 (1.00, 1.03)	0.670
GG/GA[c]	349 (81.0)	413(89.6)	**0.50 (0.34, 0.73)**	**<0.001**	**0.49 (0.33, 0.72)**	**<0.001**
rs6865292						
TT	269 (62.4)	293 (63.6)	1	–	1	–
TC	138 (32.0)	150 (32.5)	1.00 (0.75, 1.33)	0.989	1.02 (0.75, 1.34)	0.989
CC	24 (5.6)	18 (3.9)	1.45 (0.77, 2.74)	0.248	1.44 (0.76, 2.72)	0.262
TC/CC[b]	162 (37.6)	168 (36.4)	1.05 (0.80, 1.38)	0.723	1.05 (0.80, 1.38)	0.726
TT/TC[c]	407 (94.4)	443 (96.1)	0.69 (0.37, 1.29)	0.234	0.70 (0.37, 1.30)	0.257
rs4607376						
GG	155 (36.0)	160 (34.7)	1	–	1	–
GA	183 (42.5)	230 (49.9)	0.82 (0.61, 1.10)	0.190	0.82 (0.61, 1.10)	0.190
AA	93 (21.6)	71 (15.4)	1.35 (0.93, 1.98)	0.119	1.39 (0.95, 2.04)	0.092
GA/AA[b]	276 (64.1)	301 (65.3)	0.95 (0.72, 1.25)	0.695	0.95 (0.72, 1.26)	0.732
GG/GA[c]	338 (78.4)	390 (84.6)	0.66 (0.47, 0.93)	0.018	0.64 (0.46, 0.91)	0.012
rs12054797						
CC	347(80.5)	379 (82.2)	1	–	1	–
CT	77 (17.9)	77 (16.7)	1.09 (0.77, 1.55)	0.619	1.08 (0.76, 1.15)	0.683
TT	7 (1.6)	5 (1.1)	1.53 (0.48, 4.86)	0.472	1.40 (0.44, 4.46)	0.573
CT/TT[b]	84 (19.5)	82 (17.8)	1.12 (0.80, 1.57)	0.514	1.10 (0.78, 1.54)	0.598
CC/CT[c]	424 (98.4)	456 (98.9)	0.66 (0.21, 2.11)	0.487	0.73 (0.23, 2.32)	0.589

Bold values are statistically significant ($P < 0.05$)

[a] Adjusted for age, gender, smoking, drinking, and physical activity

[b] Dominant model

[c] Recessive model

Haplotype analysis on NR3C1 variations

Haplotype analysis was carried out to examine associations between SNPs that showed evidence for association in the single SNP analysis (rs2963156, rs41423247, rs10052957 and rs7701443). We identified six haplotypes with frequency greater than 5 %. Combined, these six haplotypes accounted for 90.6 % of the total haplotypes in this population. Two haplotypes (CGGA and CCAG) were significantly associated with the MetS (Table 5). The haplotype CGGA encompassing minor alleles of rs41423247 and rs7701443 was risk conferring (adjusted OR = 1.53, 95 % CI 1.06–2.20, $P = 0.023$), whereas haplotype CCAG was protective (adjusted OR = 0.30, 95 % CI 0.20–0.47, $P < 0.001$).

Discussion

We carried out a comprehensive gene-wide study to examine associations between the NR3C1 gene and MetS in a Chinese Han population. In single-marker analysis, we observed that four SNPs of the 19 markers studied were potentially associated with MetS. Haplotype analysis of SNPs associated with MetS provided evidence for associations as well. We also identified genotype/allele associations between each of the four significant associated markers and metabolic phenotypes.

The GR is a ubiquitously expressed intracellular, ligand-dependent transcription factor, which mediates the action of GCs and influences physiologic functions essential for life. As stress effectors and agents of adaptation to stress, GCs take instant part in the regulation of metabolism, homeostasis, immune reactions, and behavioral responses. Approximately 20 % of the genes expressed in human leukocytes are regulated positively or negatively by GCs [19]. Various lines of evidence indicate that variation in the NR3C1 gene can influence sensitivity to GCs in normal and diseased conditions and modify response to GCs. The stochastic nature of GC signaling pathways in association with the variable effect that NR3C1 gene mutations/polymorphisms might have on

Table 4 Association of NR3C1 gene type/alleles and metabolic phenotypes

Variable	Elevated WC		Elevated TG		Reduced HDL-C		Elevated BP		Elevated FPG	
	Adjusted OR (95 % CI)[a]	*P* value	Adjusted OR (95 % CI)[a]	*P* value	Adjusted OR (95 % CI)[a]	*P* value	Adjusted OR (95 % CI)[a]	*P* value	Adjusted OR (95 % CI)[a]	*P* value
rs2963156										
CC	1	–	1	–	1	–	1	–	1	–
CT	**1.57 (1.16, 2.14)**	**0.004**	1.24 (0.91, 2.18)	0.179	1.22 (0.88, 1.68)	0.230	**1.59 (1.17, 2.16)**	**0.003**	1.35 (1.00, 1.82)	0.052
TT	1.71 (0.83, 3.52)	0.143	2.39 (1.19, 4.80)	0.014	1.56 (0.73, 3.18)	0.223	1.94 (0.95, 4.04)	0.078	0.59 (0.28, 1.24)	0.162
CT/TT[b]	**1.59 (1.19, 2.13)**	**0.002**	1.35 (1.00, 1.81)	0.047	1.26 (0.93, 1.71)	0.143	**1.63 (1.21, 2.18)**	**0.001**	1.22 (0.92, 1.62)	0.175
CC/CT[c]	0.66 (0.32, 1.35)	0.254	0.44 (0.22, 0.89)	0.021	0.68 (0.34, 1.38)	0.283	0.62 (0.31, 1.26)	0.186	1.84 (0.88, 3.81)	0.104
rs41423247										
CC	1	–	1	–	1	–	1	–	1	–
CG	1.29 (0.97, 1.71)	0.077	1.02 (0.76, 1.36)	0.894	0.87 (0.64-1.18)	0.355	**2.14 (1.61, 2.85)**	**<0.001**	1.48 (1.12, 1.96)	0.007
GG	**2.10 (1.29, 3.43)**	**0.003**	1.09 (0.68, 1.75)	0.726	0.90 (0.54, 1.49)	0.681	**3.26 (1.95, 5.34)**	**<0.001**	1.02 (0.64, 1.62)	0.947
CG/GG[b]	1.42 (1.09, 1.85)	0.010	1.03 (0.79, 1.36)	0.813	0.87 (0.65, 1.16)	0.351	**2.31 (1.77, 3.03)**	**<0.001**	1.37 (1.05, 1.79)	0.019
CC/CG[c]	0.53 (0.33, 0.85)	0.009	0.93 (0.59, 1.46)	0.743	1.05 (0.64, 1.71)	0.856	**0.42 (0.26, 0.69)**	**<0.001**	1.16 (0.74, 1.82)	0.508
rs10052957										
GG	1	–	1	–	1	–	1	–	1	–
GA	0.83 (0.60, 1.15)	0.267	0.79 (0.56, 1.12)	0.182	1.04 (0.73, 1.48)	0.832	0.72 (0.52, 1.00)	0.049	**0.51 (0.36, 0.71)**	**<0.001**
AA	0.35 (0.12, 1.02)	0.054	0.12 (0.02, 0.88)	0.037	0.54 (0.15, 1.91)	0.339	0.17 (0.05, 0.60)	0.006	0.14 (0.03, 0.63)	0.010
GA/GG[b]	0.78 (0.58, 1.07)	0.119	0.72 (0.51, 1.01)	0.058	0.99 (0.70, 1.40)	0.966	0.65 (0.48, 0.90)	0.008	**0.47 (0.34, 0.66)**	**<0.001**
GG/GA[c]	2.75 (0.95, 7.98)	0.063	8.30 (1.09,63.44)	0.041	1.87 (0.53, 6.61)	0.332	5.55 (1.57,19.62)	0.008	6.16 (1.39,27.26)	0.017
rs7701443										
GG	1	–	1	–	1	–	1	–	1	–
GA	0.94 (0.71, 1.23)	0.667	0.69 (0.52, 0.93)	0.015	1.26 (0.92, 1.73)	0.149	0.83 (0.63, 1.11)	0.203	1.14 (0.85, 1.51)	0.385
AA	**2.22 (1.44, 3.40)**	**<0.001**	0.84 (0.55, 1.26)	0.140	**1.87 (1.23, 2.86)**	**0.004**	1.81 (1.19, 2.77)	0.006	1.53 (1.03, 2.28)	0.038
GA/AA[b]	1.15 (0.88, 1.50)	0.309	0.72 (0.55, 0.96)	0.023	1.40 (1.04, 1.88)	0.026	1.00 (0.77, 1.31)	0.998	1.22 (0.94, 1.60)	0.141
GG/GA[c]	**0.44 (0.29, 0.66)**	**<0.001**	1.00 (0.68, 1.47)	0.990	0.60 (0.41, 0.89)	0.010	0.50 (0.34, 0.75)	0.001	0.70 (0.48, 1.01)	0.059

Bold values are statistically significant ($P < 0.05$)

[a] Adjusted for age, gender, smoking, drinking, and physical activity

[b] Dominant model

[c] Recessive model

Table 5 Haplotype (rs2963156–rs41423247–rs10052957–rs7701443) frequencies of NR3C1 gene and the risk with MetS

Haplotype	Freq.	Case *N* (%)	Control *N* (%)	χ^2	*P* value	OR (95 % CI)
CCGG	0.434	178.0 (41.3)	208.8 (45.3)	2.809	0.094	0.85 (0.71–1.03)
CCGA	0.161	74.1 (17.2)	69.2 (15)	1.601	0.206	1.18 (0.91–1.52)
TGGA	0.096	46.1 (10.7)	40.1 (8.7)	2.058	0.151	1.26 (0.92–1.73)
CGGG	0.077	34.9 (8.1)	33.7 (7.3)	0.341	0.559	1.11 (0.78–1.57)
CGGA	0.071	36.6 (8.5)	26.3 (5.7)	5.137	**0.023**	**1.53 (1.06–2.20)**
CCAG	0.067	13.8 (3.2)	45.6 (9.9)	31.98	**1.55E–8**	**0.30 (0.20–0.47)**

Bold values are statistically significant ($P < 0.05$)

glucocorticoid signal transduction indicates that alterations in GR action may have important implications for many critical biological processes [20].

In our previous study conducted in a healthy Chinese population, five markers including rs2963156, rs41423247 (*Bcl*I), rs10052957 (*Tth111*I), rs7701443, and rs17209251 were associated with cardiovascular risk factors [17]. In the present case–control study, four of the five markers were found to have a statistically significant effect on MetS risk. These findings suggest that variations across the NR3C1 gene may play a role in susceptibility to cardiovascular disease.

In single locus analysis, GG of *Bcl*I was found to contribute an independent increased risk for MetS compared with CC. Increased WC and blood pressure were associated with GG genotype and G allele of *Bcl*I, respectively. These are consistent with our results of previous study that GG homozygote is associated with increased BMI. van Rossum [9] reported that in middle-aged subjects, the G allele of this *Bcl*I polymorphism was associated with increased abdominal obesity. However, at baseline, they show that female homozygous GG carriers tend to have more subcutaneous fat than CC carriers and CG carriers. It might explain why they did not find an even greater increase in GG carriers than in CG carriers during follow-up. Thus, in his study, the GG carriers might already have been slightly fatter at preadolescent age. *Bcl*I has been reported to be associated with human higher blood pressure values and/or higher prevalence of hypertension in Caucasian and Indian populations [21]. In a report by Panarelli et al. [22], increased skin vasoconstriction was observed in homozygous G allele carriers after injection with budesonide, a synthetic GC, which suggests increased in vivo sensitivity to GCs. Contrasting data have been reported about the *Bcl*I polymorphism with respect to its association with body composition. In the most studies, the *Bcl*I polymorphism shows clear associations with increased sensitivity to GCs. *Bcl*I polymorphism significantly affects the process of alternative NR3C1 gene splicing and within that mechanism increases the sensitivity to GCs [23].

For SNP rs10052957 (*Tth111*I), the A allele was found to correlate with decreased risk of MetS, and this association may be partially due to their effects on glucose metabolism. The site of the *Tth111*I polymorphism is located in the intron close to the initiation site and 3807 pb above the first site where transcription starts in exon 2 [23]. It causes G>A substitution in the promoter region. It is considered that the observed resistance to GCs and the normal metabolic profile of *Tth111*I SNP carriers is due to ER22/23EK polymorphism that is present in them [14, 24]. ER22/23EK polymorphism was associated with a relative resistance to GCs. In line with this, ER22/23EK carriers had lower cholesterol levels as well as a better insulin sensitivity [13, 25]. However, we did not find variation of ER22/23EK in the sample, as well as N363S, A3669G, and D401H.

In single locus analysis, we also found that SNP rs7701443 AA and rs2963156 CT, CT/TT contribute independent increased risk for MetS compared with GG and CC ,respectively. AA genotype of rs7701443 is associated with elevated WC and reduced HDL-C. T allele of rs2963156 is associated with elevated WC and BP. These results indicate that polymorphism of rs7701443 and rs2963156 may be associated with increased sensitivity to GCs, which was indicated in children with Crohn's disease in Krupovesa's study [26]. The exact molecular mechanisms of how rs7701443 and rs2963156 variants affect MetS are unknown and require further investigation.

In haplotype analysis, we found that haplotype CGGA had 1.53-fold risk, while haplotype CCAG was a protective factor for MetS. Not surprisingly, the differences between haplotype CGGA and CCAG were associated with rs41423247 C/G, rs10052957 G/A, and rs7701443 G/A alleles. All of these risk alleles (rs41423247/G, rs10052957/G, and rs7701443/A) contributed to the risk haplotype of CGGA, while the protective alleles (rs41423247/C rs10052957/A, and rs7701443/G) contributed to the haplotype CCAG. The presence of the rs2963156 C allele in both the haplotypes indicated that rs2963156 did not influence the effects of the haplotypes on the risk of developing MetS.

In conclusion, our findings suggest that variations across the NR3C1 gene may play a role in susceptibility to MetS. The exact molecular mechanisms of how variants affect MetS are need to be carried out to assess the implications of these associations. In clinical practice, it might be useful to screen for the presence of these NR3C1 gene variants, in order to determine an individual's preventive and therapeutic intervention for cardiovascular diseases.

Acknowledgments This study was supported by the National Natural Science Foundation (81102208), the Beijing Natural Science Foundation (7133229), the National Science and Technology Support Program (2012BAI37B03), and the Importation and Development of High-Caliber Talents Project of Beijing Municipal Institutions (CIT&TCD201304181).

Conflict of interest There was no conflict of interest in this study.

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