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Polymorphism in exon 4 of the human 3β-hydroxysteroid dehydrogenase type I gene (*HSD3B1*) and blood pressure

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Abstract

There is growing evidence to the effect that steroid hormones are associated with a complex phenotype of metabolic abnormalities usually referred to as the metabolic syndrome. The 3β-hydroxysteroid dehydrogenases/$\Delta^{4,5}$-isomerase (3β-HSD) is crucial to the biosynthesis of hormonal steroids, including aldosterone, cortisol, and testosterone. The objective of the present study was to examine the potential impact of a T → C substitution at codon Leu^{338} of the type I (*HSD3B1*) 3β-HSD gene on obesity, circulating hormones, and estimates of insulin, glucose, and lipid metabolism as well as blood pressure in 284 unrelated Swedish men born in 1944. The subjects were genotyped by using PCR amplification of exon 4 of the *HSD3B1* gene followed by digestion with the restriction enzyme *Bgl*II. The frequency of allele T was 0.44 and that of allele C 0.56. Homozygotes for the C allele ($n = 75$) had significantly ($P < 0.05$) higher mean systolic and diastolic blood pressures compared to both heterozygotes ($n = 143$) and homozygotes for the T allele ($n = 45$). In addition, the C allele was significantly ($P = 0.018$) more frequent among subjects with grade 1 hypertension (>140/90 mm Hg) compared to normotensive (<130/85 mm Hg) subjects. These results were all adjusted for the potential confounding effect of body mass index (BMI) and waist-to-hip ratio (WHR). Other measurements such as BMI, WHR, abdominal sagittal diameter, salivary cortisol, total testosterone, serum leptin, fasting insulin and glucose, and serum lipids were not different across the *HSD3B1* genotype groups. In conclusion, a T → C polymorphism at codon Leu^{338} of exon 4 of the *HSD3B1* gene is associated with elevated systolic and diastolic blood pressures. The pathogenic mechanism underlying this association is, however, uncertain from the present data and further studies are warranted.

Keywords: Blood pressure; Genes; Obesity; Polymorphism; Steroids

There is a clustering of several risk factors, including obesity, hypertension, glucose intolerance, diabetes mellitus, and hyperlipidemia, which is observed more frequently than by chance alone. This has led to the suggestion that these represent a single syndrome which is referred to as the metabolic syndrome [1]. A growing body of evidence suggests that glucocorticoid secretion and/or metabolism is associated with this complex phenotype [2,3]. In addition to glucocorticoid, a relative androgen deficiency in men appears to underlie the pathogenesis of different components of this syndrome [4].

The 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$-isomerase (3β-HSD) enzyme is crucial to the biosynthesis of hormonal steroids, among others aldosterone, cortisol, and testosterone [5]. This enzyme is expressed in the placenta, adrenals, testes, and in peripheral tissues such as muscle and adipose tissue [5]. There are two genes located on chromosome 1p13.1, which encode two different 3β-HSD isoenzymes [5]. The structures of type I (*HSD3B1*) and type II (*HSD3B2*) 3β-HSD genes consist of four exons included within a DNA sequence of 7.8 kb [6]. To date, more than 30 mutations have been identified in the *HSD3B2* gene [7]. In addition, Rhéaume et al. [8] demonstrated a *Bgl*II restriction fragment length polymorphism (RFLP) due to a substitution of T by C in exon 4 in the *HSD3B1* gene and demonstrated linkage disequilibrium between this RFLP and mutations in the *HSD3B2* gene [9].

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To our knowledge, mutations in the *HSD3B1* gene have not yet been found to be associated with any specific disease or symptoms. In the present study, we tested the hypothesis that a T → C substitution at codon Leu[338] in exon 4 of the *HSD3B1* gene is associated with obesity, circulating hormones, including salivary cortisol, fasting insulin and glucose, and lipid metabolism as well as resting blood pressure.

Subjects and methods

Subjects. In the present study, we recruited the subjects from an ongoing cohort study of men born in 1944 [10,11]. The study was initiated in 1992. Based on the self-measured waist-to-hip ratio (WHR) the following three subgroups, each of 150 men, were selected for further studies: the lowest ($\leqslant 0.885$) and the highest values ($\geqslant 1.01$) as well as men around the arithmetic mean (0.94–0.96). We examined these men in 1995 at the age of 51 yr and 284 (63%) volunteered to participate [12,13]. Nine subjects refused to be involved in the genetic studies and in 12 subjects the amount of blood samples was not sufficient to conduct any genetic analysis. All men gave written informed consent before participating in the study, which was approved by the Göteborg University Ethics Committee.

Phenotypic measurements. Body mass index (BMI, kg/m^2), WHR, and abdominal sagittal diameter were measured, as described previously [12,13]. Salivary cortisol was measured repeatedly over a random working day as described previously [12]. Endocrine measurements, beside cortisol, included testosterone, insulin-like growth factor I, and leptin as described previously [12,13]. Insulin, glucose, triglycerides, total, and high- and low-density lipoprotein cholesterol were measured in the overnight fasting state as described previously [12,13]. The serum lipids were determined by an enzymatic procedure in a Cobas Fara II (Roche Molecular Biochemicals, Mannheim, Germany). Two blood pressure readings were recorded on the right arm with the participants sitting, using a random-zero mercury sphygmomanometer, after 5 min of resting, with the auscultation site at heart level, a peak inflation level of 30 mm Hg above radial pulse disappearance, and a cuff-deflation rate of 2–3 mm Hg/s. Values were recorded to the nearest even digit. Heart rate was recorded simultaneously and systolic and diastolic blood pressures were calculated as the means of the two measurements.

Genotyping. Genotyping of the T(TG) → C(TG) sequence variation in exon 4 was carried out by PCR-RFLP. Genomic leukocyte DNA (150 ng in a final volume of 10 μL) was amplified by PCR using the following primers: 5′-AAG TGT TGG AAA GTT CTC CAC TGT T-3′ and 5′-GTG CCC TTG TCA CTT TCT GTA TGA G-3′ [8]. The primers amplified a product of 575 bp. The PCR conditions were: an initial denaturation step at 95 °C for 3 min, followed by 29 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, and extension at 70 °C for 1 min, with a final extension of 10 min at 72 °C. The PCR product was digested at 37 °C overnight with 6 U of the restriction enzyme *Bgl*II (New England Biolabs, Beverly, MA, USA), creating two fragments of 204 and 371 bp when the T–C sequence variation is present. The fragments were separated on a 1.5% agarose gel and visualized under UV with ethidium bromide.

Statistical analysis. All statistical analyses were performed using SPSS for Windows, release 10.0 (SPSS, Chicago, IL). The *P* values are two-sided throughout and a $P < 0.05$ was considered significant. The results are presented as means and standard deviation (SD). Data comparisons were carried out with the General Linear Model, with genotype as independent factor and BMI and WHR as covariates. All *P* values were adjusted for multiple tests by using the Spjotvoll–Stoline post hoc correction [14]. Tests of independence in a $2 \times K$ table were performed with the Fisher–Freeman–Halton exact test [15].

Results

The frequency of allele T was 0.44 and that of allele C 0.56. The observed genotype frequencies were 19.3%, 49.3%, and 31.4% for T/T, T/C, and C/C, respectively. Genotype frequencies were in a Hardy–Weinberg equilibrium.

Table 1 shows the phenotypic characteristics of the *Bgl*II *HSD3B1* genotypes. Homozygotes for the C allele had significantly ($P < 0.05$) higher systolic and diastolic

Table 1
Differences in anthropometric, endocrine, metabolic, and circulatory measurements by genotype of the T/C polymorphism at codon Leu[338] of the *HSD3B1* gene

	Genotypes		
	T/T ($n = 45$)	T/C ($n = 143$)	C/C ($n = 75$)
Body mass index (kg/m^2)	25.8 (3.5)	26.0 (4.3)	26.8 (3.4)
Waist-to-hip ratio	0.93 (0.09)	0.94 (0.07)	0.94 (0.06)
Abdominal sagittal diameter (cm)	22.3 (3.2)	22.4 (3.9)	23.5 (3.2)
Diurnal cortisol secretion (nmol/L)	7.0 (2.0)	7.4 (3.4)	8.0 (4.9)
Testosterone (nmol/L)	19.4 (5.2)	20.1 (5.8)	19.1 (5.0)
Insulin-like growth factor I (μg/L)	204.2 (58.4)	207.8 (66.4)	202.1 (66.6)
Leptin (μg/L)	6.0 (4.6)	6.3 (4.7)	6.1 (3.2)
Fasting insulin (mU/L)	12.2 (7.7)	12.3 (10.9)	13.6 (12.9)
Fasting glucose (mmol/L)	4.4 (1.0)	4.6 (0.9)	4.6 (1.1)
Triglycerides (mmol/L)	1.7 (0.9)	1.8 (1.1)	1.9 (1.0)
Total cholesterol (mmol/L)	5.9 (1.0)	6.1 (1.1)	6.4 (0.9)
High-density lipoprotein cholesterol (mmol/L)	1.2 (0.3)	1.2 (0.3)	1.3 (0.4)
Low-density lipoprotein cholesterol (mmol/L)	3.9 (1.0)	4.1 (1.1)	4.2 (0.9)
Systolic blood pressure (mm Hg)	125.0 (14.1)[a]	128.4 (17.5)[a]	133.8 (18.8)[b]
Diastolic blood pressure (mm Hg)	80.6 (9.2)[a]	82.9 (10.6)[a]	86.0 (10.5)[b]
Heart rate (beats/min)	67.4 (10.1)	69.5 (11.0)	69.1 (9.8)

Values (means ± SD) with different letters are significant at the 0.05 level. Data were adjusted for BMI and WHR when appropriate.

Table 2
Distribution of the *Bgl*II *HSD3B1* genotypes and allele frequencies in normotensive and grade 1 (mild) hypertensive subjects

	<130/85 mm Hg ($n = 115$)	>140/90 mm Hg ($n = 39$)
Genotype		
T/T	26 (22.6%)	4 (10.2%)
T/C	68 (59.1%)	20 (51.3%)
C/C	21 (18.3%)	15 (38.5%)
P	0.027	
Allele frequency		
T allele	120 (52.2%)	28 (35.9%)
C allele	110 (47.8%)	50 (64.1%)
P	0.018	

blood pressures compared to both heterozygotes and homozygotes for the T allele. These results were all adjusted for the potential confounding effect of BMI and WHR. Other measurements such as BMI, WHR, abdominal sagittal diameter, salivary cortisol, total testosterone, insulin-like growth factor I, serum leptin, fasting insulin and glucose, and serum lipids were not different across the *HSD3B1* genotype groups.

Table 2 shows the distribution of the *Bgl*II *HSD3B1* genotypes and allele frequency in normotensive (<130/85 mm Hg) and grade 1 (mild) hypertensive (>140/90 mm Hg) subjects [16]. The C/C genotype was significantly ($P = 0.027$) more frequent among hypertensive subjects. In addition, the C allele was significantly ($P = 0.018$) more frequent in the same group compared to the normotensive subjects.

Discussion

The examined men were selected from an ongoing cohort study and 80% volunteered to participate in the first part of the study. The second part, which was laboratory-based, attracted fewer participants, but the non-responders showed a structure, similar to the responders in such a way that the selection bias is probably negligible [12,13].

The findings reported here are, to our knowledge, the first showing a relationship between a polymorphism in the *HSD3B1* gene and blood pressure in humans. The main findings are that homozygotes for the C allele had higher systolic and diastolic blood pressures compared to other *Bgl*II *HSD3B1* genotypes. The T/T homozygoteshad systolic/diastolic blood pressures that were 5.4/3.1 mm Hg lower in comparison with the heterozygotes and 8.8/5.4 mm Hg in comparison with the homozygotes for the C allele. These differences were independent of BMI and body fat distribution. Moreover, subjects with grade 1 hypertension had a significantly different distribution of the *Bgl*II *HSD3B1* genotypes than normotensive subjects. In addition, the C allele was significantly more frequent in grade 1 hypertensive subjects.

Hypertension is frequently found in obesity and together with other associated metabolic abnormalities [4]. The *Bgl*II *HSD3B1* polymorphism was, however, associated only with blood pressure in the present study.

The activities of the type I and type II 3β-HSD isoenzymes are essential for the formation of all classes of steroids. The structure of the enzymes of the 3β-HSD gene family has been characterized only recently in humans and little is known about the structure–function relationships of type I and type II 3β-HSD isoenzymes [5,17]. From the present data, we conclude that a T → C substitution in exon 4 of the *HSD3B1* gene is unlikely to be associated with either salivary cortisol or serum testosterone (Table 1), although a trend towards elevated cortisol levels in C/C subjects was found. Moreover, we found no statistically significant association between the *Bgl*II polymorphism of the *HSD3B1* gene and obesity (BMI) and body fat distribution (WHR and abdominal sagittal diameter).

Blood pressure is determined by the product of cardiac output, intravascular volume, and peripheral resistance. Because hormones are involved in blood pressure regulation and affect these parameters, hypertension is a prominent feature of certain adrenal enzymatic abnormalities [18]. Aldosterone, the most important mineralocorticoid, regulates electrolyte excretion and intravascular volume mainly by increasing sodium resorption and potassium excretion into urine [19]. Excess secretion of aldosterone or other mineralocorticoids or abnormal sensitivity to mineralocorticoids may result in hypertension. Such conditions usually have a genetic basis [18]. Thus, one might speculate that the pathogenic mechanism underlying the observed blood pressure elevation associated with the *Bgl*II *HSD3B1* polymorphism might be an increased secretion of aldosterone or other mineralocorticoids. However, to what extent this polymorphism is associated with mineralocorticoids secretion is uncertain in the absence of such measurements.

One might consider the T allele to be protective against the development of hypertension. The T/T genotype is the least frequent of the genotypes. A similar phenomenon has recently been found for an allelic variant of the angiotensinogen gene, which is also rare and protective against the development of hypertension [20].

In summary, we have shown that a sequence variation in exon 4 of the *HSD3B1* gene is related to elevated systolic and diastolic blood pressures. Moreover, we found that the C allele was associated with grade 1 hypertension (>140/90 mm Hg). Since all statistical analyses were adjusted for multiple tests, and for BMI and estimates of central obesity, we find it unlikely that these findings are due to chance alone. However, the underlying pathogenic mechanism is uncertain from the present data and further studies are warranted.

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