

BRIEF COMMUNICATION

–344C/T Variant in the Promoter of the Aldosterone Synthase Gene (*CYP11B2*) Is Associated With Metabolic Syndrome in Men

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Background: The –344C/T variant in the promoter of the aldosterone synthase gene (*CYP11B2*) has been associated with hypertension and may influence glucose homeostasis and body mass in humans. We assessed the association between this genetic variant and metabolic syndrome in a large sample of European population.

Methods: Eight hundred two male/female couples, recruited in the framework of the IMMIDIET study, a survey on cardiovascular risk in Italy, UK, and Belgium, had standardized measurements of body mass index, waist circumference, blood pressure (BP), serum total and HDL-cholesterol, triglycerides, and glucose and were genotyped for the –344C/T variant of *CYP11B2*. Metabolic syndrome was defined according to the International Diabetes Federation criteria.

Results: The prevalence of the metabolic syndrome was 23.9% in men and 14.0% in women. The C allele of

the variant was associated with metabolic syndrome in men ($P = .002$) but not in women. At logistic regression analysis, the odds ratio of metabolic syndrome increased progressively with the number of copies of the C allele (CT: 1.54, 95% CI from 1.01 to 2.35; CC: 2.25, 95% CI from 1.38 to 3.66) as compared with the TT homozygotes, taken as reference genotype.

Conclusions: The C allele of –344C/T variant of *CYP11B2* increases susceptibility to metabolic syndrome in European men, but not in women, suggesting a pleiotropic role for this gene in modulating cardiovascular risk. Am J Hypertens 2007;20:218–222 © 2007 American Journal of Hypertension, Ltd.

Key Words: Aldosterone synthase, *CYP11B2*, polymorphism, metabolic syndrome, population study.

In recent years, several studies indicated that the aldosterone synthase gene (*CYP11B2*) represents a promising candidate for high blood pressure (BP) and cardiovascular disease.¹ *CYP11B2*, which encodes the aldosterone synthase enzyme, is located in chromosome 8 in men near the highly homologous (approximately 95% identity in coding sequences) *CYP11B1* encoding 11-beta-hydroxylase. Among the variants at the *CYP11B2* locus, the –344C/T polymorphism in the 5' promoter region of the gene has been associated with arterial hypertension

and hypertension-related phenotypes, although with conflicting results,¹ probably attributable to gender-, age-, and ethnicity-related variations in the phenotypic expression.^{2,3} Notwithstanding the discrepancies among studies, it is in principle understandable how polymorphisms in *CYP11B2* may affect mineralocorticoid synthesis and possibly BP regulation. More surprisingly, the condition of C homozygosity for the –344C/T variant has been found associated with higher plasma glucose levels and glucose intolerance in a large population of Chinese and Japanese

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origin,⁴ suggesting a role for this locus in glucose homeostasis. In the light of these observations, it is conceivable that *CYP11B2* may play a role not only in hypertension but also in the metabolic syndrome, as previously suggested.¹ Metabolic syndrome is defined by a cluster of factors,⁵ namely central obesity, hyperglycemia, dyslipidemia, and elevated BP, which tend to aggregate in overweight or frankly obese individuals and is associated with increased cardiovascular events.⁶

Although the definition and nosological significance of the metabolic syndrome is still object of debate, there is agreement that the full expression of the syndrome depends on a complex interaction between genetic determinants (still largely unknown) and acquired factors related mainly to lifestyle and dietary habits.⁷

In the present study we adopted the definition of metabolic syndrome recently released by the International Diabetes Federation (IDF)⁵ to explore for the first time the association between the $-344C/T$ polymorphism of the *CYP11B2* and metabolic syndrome in a large sample of European men and women, recruited in the framework of the IMMIDIET* project.⁸

Methods

Study Population

The IMMIDIET project is a population-based cross-sectional study designed to investigate gene-environment interaction in relation to cardiovascular risk in married healthy couples from Italy, Belgium, and England (for more information, see http://www.moli-sani.org/progetti/immidiet_site/welcome.html).^{8,9} The couples were randomly recruited from general practice in southeast England ($n = 263$), the Flemish territory of Belgium ($n = 268$), and the Abruzzo region of Italy ($n = 271$). Exclusion criteria for all groups were: history of cardiovascular disease (acute myocardial infarction, stable and unstable angina, stroke, transient ischemic attack, peripheral arterial disease), known diabetes (types 1 and 2), familial hypercholesterolemia, malignancies, chronic diseases like heart, liver, or renal failure, defined coagulation deficiency, hypo/hyperthyroidism, and epilepsy. Data were collected using a well-standardized questionnaire that included items on medical history, hypertension and dyslipidemia, family history of myocardial infarction, use of any medication, cigarette smoking, physical activity, and socioeconomic status. Nutrient and energy intakes were evaluated by semiquantitative food frequency questionnaires. The study was approved by the ethics committees of all participating institutions. All study participants agreed to give blood samples for DNA analysis and biochemical measurements, by written informed consent. Participating couples attended their general practitioner's clinic for the screening

visit performed by trained physicians and nurses of the IMMIDIET staff.

Blood pressure was measured with an automatic device (OMRON-HEM-705CP; Omron Health Care, Inc., Bannockburn, IL) after the subject had been resting in the seated position for 10 min and before blood sampling. Systolic and diastolic BP were taken three times, with 2-min intervals between measurements and the average of the last two readings was used for the analyses. The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2).

Biochemical Assays

Blood samples were obtained from patients who had been fasting overnight. The measurements of serum lipids and blood glucose were performed by automated analyzer (Cobas-Mira-Plus; Roche, Milan, Italy). Plasma insulin levels were determined by ELISA kit (DAKO Insulin, DAKO Ltd., Ely, UK) using a completely automated ELISA analyzer (ETI-STAR, DiaSorin S.p.A, Saluggia, Italy). The homeostasis model assessment of insulin resistance (HOMA) index was calculated as follows: Fasting insulin (mU/L) \times Fasting blood glucose (mmol/L)/22.5.¹⁰

Metabolic syndrome was defined according to the recent definition released by the IDF for European men and women,⁵ that is, waist circumference ≥ 94 cm in men or ≥ 80 cm in women, plus any two of the following four factors: increased triglycerides (TG) level, ≥ 1.7 mmol/L (150 mg/dL), or specific treatment for this lipid abnormality; reduced HDL cholesterol, < 1.03 mmol/L (40 mg/dL) in men or < 1.29 mmol/L (50 mg/dL) in women, or specific treatment for this lipid abnormality; increased BP, systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg, or treatment of previously diagnosed hypertension; increased fasting plasma glucose (FPG) ≥ 5.6 mmol/L (100 mg/dL), or previously diagnosed type 2 diabetes.

Genotyping

The DNA samples were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for $-344C/T$ variant of *CYP11B2* according to Russo et al.²

Statistical Analysis

Data are presented as mean \pm SD. All statistical analyses were performed separately on men and women. Two-tailed P values $< .01$ were considered statistically significant.

Because the distribution of HDL-cholesterol, triglyceride, and insulin levels deviated significantly from normal, they were normalized by log transformation. Log-transformed values were used in the analysis, as appropriate. Standard statistical genetic methods were used to verify that the assumption of Hardy-Weinberg equilibrium was appropriate. Differences in allele frequency were determined by χ^2 statistics. Differences in phenotypic variables between

*IMMIDIET = Dietary habit profile in European communities with different risk of myocardial infarction: the impact of migration as a model of gene-environment interaction.

affected individuals and controls were assessed by two-sided *t* test. The association between $-344C/T$ genotypes and prevalence of metabolic syndrome was first assessed by χ^2 statistics and then compared by logistic regression analysis adjusting for covariates (age, national origin, alcohol intake, and menopausal status). Odds ratios (OR) and their 95% confidence intervals (CI) were also calculated.

The population sample provided at least 90% power to detect at $P < .05$ a 15% difference in the prevalence of metabolic syndrome between *CC* and *TT* homozygotes, in men and women separately. Statistical analyses were performed with the SPSS statistical software package (SPSS 11.0; Chicago, IL).

Results

The $-344C/T$ polymorphism was genotyped in a total of 1604 individuals. The genotyping success rate was 96%. A 10% random sample was genotyped twice in a blinded fashion without any discrepancies between genotype score. The distribution of genotypes was in Hardy-Weinberg equilibrium. The frequency of the *C/T* alleles in the entire population was 45/55% in men and 46/54% in women (men: *CC* 159; *CT* 376; *TT* 231; women: *CC* 142; *CT* 402; *TT* 220). There was no statistically significant difference in allelic distribution among populations and between men and women.

The clinical characteristics of the subjects according to gender and metabolic syndrome status are shown in Table 1. The prevalence of metabolic syndrome was higher in men (23.9%) than in women (14.0%). The BMI, waist circumference, serum total cholesterol, triglycerides, glucose,

plasma insulin, and BP were higher and HDL-cholesterol was lower in both men and women with metabolic syndrome than in those without (controls). Individuals with metabolic syndrome were also older than controls. The $-344C/T$ genotype frequency in cases and controls is shown in Table 1. In men, the genotype distribution was significantly different between cases and control, the prevalence of metabolic syndrome increasing progressively and significantly through $-344C/T$ genotypes (*TT*: 17.3%; *CT* 24.2%; *CC*: 32.7%; $P = .002$). In contrast, there was no evidence of any association between the $-344C/T$ polymorphism and metabolic syndrome in women.

The association between the $-344C/T$ polymorphism and metabolic syndrome was further explored by logistic regression analysis, using the *TT* carriers as the reference group and adjusting for age, national origin, alcohol consumption (expressed as percent of total calories), and menopausal status. The odds ratio of metabolic syndrome was 1.54 (95% CI from 1.01 to 2.35) and 2.25 (95% CI from 1.38 to 3.66) in men bearing the *CT* and the *CC* genotypes, respectively. In contrast, there was no evidence of association between the variant and metabolic syndrome in women.

When the relationship between the $-344C/T$ variant and any single component of metabolic syndrome was examined separately, higher prevalence of high plasma glucose levels was found in the male carriers of the *C* allele (*CC* 13.2%, *CT* 7.7%, *TT* 5.2%, $P = .005$), whereas higher, although not statistically significant, prevalence of abdominal obesity (*CC* 61.0%, *CT* 55.4%, *CC* 53.4%) and low HDL levels (*CC* 27.7%, *CT* 23.8%, *TT* 22.3%) was observed in men.

Table 1. Characteristics of the individuals with and without metabolic syndrome

Variable	Men		Women	
	Controls	MS	Controls	MS
N (%)	583 (76.1)	183 (23.9)	666 (86.0)	108 (14.0)
Age (y)	46.9 ± 7.8	49.0 ± 7.5†	44.3 ± 7.8	48.5 ± 6.7†
BMI (kg/m ²)	26.4 ± 3.3	30.2 ± 3.4†	25.2 ± 4.5	30.3 ± 5.2†
Waist (cm)	93.4 ± 9.1	104.6 ± 8.0†	83.4 ± 10.5	95.9 ± 10.5†
Total cholesterol (mmol/L)	5.66 ± 0.95	6.08 ± 1.08†	5.50 ± 0.95	5.84 ± 1.05†
HDL-cholesterol (mmol/L)	1.30 ± 0.31	1.06 ± 0.25†	1.54 ± 0.34	1.17 ± 0.26†
TG (mmol/L)	1.28 ± 0.75	2.40 ± 1.21†	0.96 ± 0.44	1.93 ± 0.90†
FBG (mmol/L)	4.50 ± 0.65	5.07 ± 1.47†	4.31 ± 0.61	4.78 ± 1.54†
Insulin (pmol/L)	39.1 ± 26.8	63.2 ± 37.3†	35.5 ± 23.7	60.7 ± 37.7†
HOMA	1.32 ± 0.94	2.39 ± 1.57†	1.17 ± 0.87	2.23 ± 1.67†
SBP (mm Hg)	126.4 ± 14.6	137.5 ± 17.0†	114.0 ± 15.0	132.3 ± 18.9†
DBP (mm Hg)	80.1 ± 9.2	88.2 ± 9.6†	73.3 ± 8.9	83.4 ± 9.4†
<i>CYP11B2</i> genotype frequency in metabolic syndrome and controls*				
<i>CC</i>	107 (67.3)	52 (32.7)‡	135 (88.8)	17 (11.2)
<i>CT</i>	285 (75.8)	91 (24.2)‡	342 (85.1)	60 (14.9)
<i>TT</i>	191 (82.7)	40 (17.3)‡	189 (85.9)	31 (14.1)

BMI = body mass index; DBP = diastolic blood pressure; FBG = fasting blood glucose; MS = metabolic syndrome; SBP = systolic blood pressure; TG = triglycerides.

Values are mean ± SD.

* Values are n (%); † $P < .001$ by two-sided *t* test; ‡ $P = .002$ by χ^2 test.

Finally, no differences in plasma potassium levels were observed among genotypes (plasma potassium, in millimoles per liter: men: *CC* 4.0 ± 0.4 , *CT* 4.0 ± 0.3 , *TT* 4.0 ± 0.4 ; women: *CC* 4.0 ± 0.3 , *CT* 4.0 ± 0.3 , *TT* 4.0 ± 0.3).

Discussion

The present study reports for the first time the association between the *-344C/T* variant of the 5' promoter region of *CYP11B2* and the metabolic syndrome in a large and well-characterized European population sample. This association was found in men but not in women, suggesting a gender-related susceptibility of the complex phenotype "metabolic syndrome" to this genetic variant. The discussion whether the metabolic syndrome is a distinct nosologic entity or merely a cluster of separate factors⁵ is far beyond the objective of this study. Nevertheless, there is strong evidence to indicate that diverse genetic factors modulate an individual's predisposition for the development of the quantitative phenotypes, including BP, BMI, waist circumference, HDL-cholesterol, triglycerides, glucose, and insulin levels, underlying the syndrome itself.⁷ It is conceivable that the prime candidate genes for metabolic syndrome are those able to simultaneously regulate different steps of the processes leading to full expression of the syndrome itself.⁷ The hypothesis that adrenal glomerulosa products and activity may interface with the factors included in the metabolic syndrome is not new¹¹ and has been recently confirmed by the observations by Fallo et al¹² and by Bochud et al,¹³ both focusing on the role of aldosterone excess on the phenotypic expression of metabolic syndrome.

In particular, there is circumstantial evidence that genetic variants of the *CYP11B2*, encoding for the aldosterone synthase, may be involved not only in mineralocorticoid synthesis and BP regulation,¹ but also in glucose homeostasis.⁴ This raises the possibility that the *CYP11B2* locus may be important for the phenotypic expression of the metabolic syndrome, either directly or through interaction with the highly homologous adjacent *CYP11B1*, encoding for 11-beta-hydroxylase,¹⁴ although this hypothesis is far from being demonstrated.¹⁵ It is quite difficult at the moment to provide a potential mechanism by which this variant could promote susceptibility to the metabolic syndrome. Recent data suggested that the *Lys173Arg* polymorphism in the coding region of *CYP11B2*, which is in complete linkage disequilibrium with *-344C/T*, may differentially regulate the expression of the gene in the adrenal gland, thus confirming the functional relevance of the variant.¹⁶ The putative effect of allelic variations of *CYP11B2* not only on the biosynthesis of mineralocorticoids but also on that of glucocorticoids has been recently shown,¹⁴ further confirming the hypothesis that variations at the *CYP11B2* locus may be markers of variations at the adjacent *CYP11B1* locus, which in turn may be associated to abnormalities in β -hy-

droxylation and glucocorticoid synthesis.¹⁴ Alternatively, a direct effect of genetic variations at the *CYP11B2* locus may influence aldosterone production and action, in turn affecting susceptibility to the metabolic syndrome, as recently suggested.^{12,13} Notwithstanding which hypothesis is true, the biological plausibility of the association of *-344C/T* with the metabolic syndrome lies on the experimental evidence of a close association between disturbances of the adrenocortical axis and most features of the metabolic syndrome.¹⁷

The present study has some strengths and limitations. It was conducted on a large sample of couples randomly recruited from general practice in three European countries and represents to our knowledge one of the largest genetic association studies on metabolic syndrome. The analyses were performed separately in men and women and the particular protocol of the IMMIDIET study, carried out in cohabiting partners, reduced at the minimum the impact of environmental factors on sex-related differences, often observed in association studies. Moreover, the allelic effect was dose-dependent, the strength of the association increasing with the number of *C* allele copies. Finally, our data confirmed the previous observation of higher blood glucose levels in the carriers of the *C* allele of *-344C/T*⁴ and were also in agreement with the observation by Fallo et al¹² of higher plasma glucose levels in patients with primary aldosteronism.

The study has limitations as well, some of which are inherent to the use of genetic association for the study of multifactorial common diseases¹⁶ and some others are related to the methodologic constraints posed by a large-scale epidemiologic investigation such as IMMIDIET. Because the IMMIDIET cohort comprises only white participants, our conclusions cannot be generalized to populations other than white European men and women. Although the *-344C/T* genotype has been independently and consistently associated with diverse cardiovascular phenotypes,¹ the identification of risk haplotypes within *CYP11B2* (or the adjacent *CYP11B1*) would significantly increase the discriminatory power and the physiologic relevance of this candidate locus. Finally, the measurement of plasma aldosterone or of plasma and urinary metabolites of cortisol precursors (11-deoxycortisol, tetrahydro-11-deoxycortisol) might have been useful to gain further insight into the possible link between *CYP11B2* and the development of the metabolic syndrome.¹²⁻¹⁴

In conclusion, the present study showed for the first time that the *C* allele of *-344C/T* variant of *CYP11B2* increases susceptibility to the metabolic syndrome in European men, but not in women. Our data are consistent with the hypothesis that components of the renin-angiotensin-aldosterone system, with pleiotropic effects on different metabolic pathways, may have a role in the phenotypic expression of the metabolic syndrome.¹⁸

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