Brief Genetic Analysis

Molecular Screening of the 11β-HSD1 Gene in Men Characterized by the Metabolic Syndrome

Julie Robitaille,† Charles Brouillette,*‡ Alain Houde,* Jean-Pierre Després,†§ André Tchernof,*†¶ and Marie-Claude Vohl*†*

Abstract

ROBITAILLE, JULIE, CHARLES BROUILLETTE, ALAIN HOUDE, JEAN-PIERRE DESPRÉS, ANDRÉ TCHERNOF, AND MARIE-CLAUDE VOHL. Molecular screening of the 11β-HSD1 gene in men characterized by the metabolic syndrome. *Obes Res.* 2004;12:1570–1575.

Adipose tissue type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1), which generates hormonally active cortisol from inactive cortisone, has been shown to play a central role in adipocyte differentiation and abdominal obesity-related metabolic complications. The objective was to investigate whether genetic variations in the human *11β-HSD1* gene are associated with the metabolic syndrome among French-Canadian men. We sequenced all exons, the exon-intron splicing boundaries, and 5′ and 3′ regions of the human *11β-HSD1* gene in 36 men with the metabolic syndrome, as defined by the National Cholesterol Education Program-Adult Treatment Panel III, and two controls. Three intronic sequence variants were identified: two single-nucleotide polymorphisms in intron 3 (g.4478T>G) and intron 4 (g.10733G>C) and one insertion in intron 3 (g.4437-4438insA). The relative allele frequency was 19.6%, 22.1%, and 19.6% for the g.4478G, g.10733C, and g.4438insA alleles, respectively. One single-nucleotide polymorphism was identified in exon 6 (c.744G>C or G248G). The frequency of the c.744C allele was only 0.46% in a sample of 217 men. Variants were not associated with components of the metabolic syndrome except for plasma apolipoprotein B levels. In conclusion, molecular screening of the *11β-HSD1* gene did not reveal any sequence variations that can significantly contribute to the etiology of the metabolic syndrome among French-Canadians.

Key words: *11β-HSD1*, susceptibility gene, abdominal obesity, metabolic syndrome

The increased morbidity related to obesity may be largely accounted for by the presence of several risk factors defining the metabolic syndrome. The National Cholesterol Education Program (NCEP)[1]-Adult Treatment Panel III (ATPIII) has proposed to characterize the metabolic syndrome as the presence of three or more of the following components: abdominal obesity, high circulating concentrations of triglycerides, hypoalphalipoproteinemia, hypertension, and high fasting plasma glucose levels (1). Knowledge of factors determining the severity of the syndrome remains largely incomplete, but there is strong evidence for genetic susceptibilities in this pathophysiology (2).

Adipose tissue type 1 11β-hydroxysteroid dehydrogenase (*11β-HSD1*), which generates hormonally active cortisol from inactive cortisone, has been shown to play a central role in adipocyte differentiation and abdominal obesity-related metabolic complications (3). An accumulation of abdominal fat in the visceral compartment is observed in patients with Cushing's syndrome, suggesting that glucocorticoids play a potentially key role in the pathogenesis of visceral obesity (4). Moreover, transgenic mice overexpressing *11β-HSD1* in adipose tissue developed visceral obesity and an insulin-resistant state and hyperlipidemia, leading the investigators to suggest that *11β-HSD1* may be a common molecular etiology for visceral obesity and the metabolic syndrome (5). Thus, the gene coding for this enzyme represents a promising candidate for the metabolic

Received for review December 15, 2003.
Accepted in final form July 22, 2004.
The costs of publication of this article were defrayed, in part, by the payment of page charges. This article must, therefore, be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*Lipid Research Center and ¶Endocrinology and Molecular Oncology Research Center, Laval University Hospital Research Center, Ste-Foy, Quebec, Canada; Departments of †Food Science and Nutrition and ‡Social and Preventive Medicine, Division of Kinesiology, Laval University, Quebec, Canada; §Quebec Heart Institute, Ste-Foy, Quebec, Canada; Address correspondence to Marie-Claude Vohl, Lipid Research Center, CHUQ-CHUL, 2705 Laurier Boulevard, TR-93, Ste-Foy, Quebec, Canada G1V 4G2.
E-mail: marie-claude.vohl@crchul.ulaval.ca

[1] Nonstandard abbreviations: NCEP, National Cholesterol Education Program; ATPIII, Adult Treatment Panel III; *11β-HSD1*, 11β-hydroxysteroid dehydrogenase; SNP, single-nucleotide polymorphism; apo B, apolipoprotein B; PCR, polymerase chain reaction; CIHR, Canadian Institutes of Health Research.

Table 1. Subjects' characteristics

Variables	Mean ± SD	Range
Age (years)	42.8 ± 7.9	22.0 to 63.0
BMI (kg/m^2)	29.6 ± 4.3	18.7 to 41.0
Waist circumference (cm)	101.4 ± 11.4	67.0 to 127.7
Total cholesterol (mM)	5.19 ± 0.76	3.57 to 7.26
Low-density lipoprotein-cholesterol (mM)	3.44 ± 0.74	1.45 to 5.26
High-density lipoprotein-cholesterol (mM)	0.92 ± 0.21	0.49 to 1.87
Total cholesterol/high-density lipoprotein-cholesterol	5.89 ± 1.44	2.47 to 10.80
Triglycerides (mM)	2.22 ± 1.10	0.45 to 7.56
Diastolic blood pressure (mm Hg)	81.93 ± 7.24	63.0 to 100.0
Systolic blood pressure (mm Hg)	122.30 ± 9.58	95.0 to 157.0

syndrome. The aim of this study was to investigate whether genetic variants in the human *11β-HSD1* gene are associated with the metabolic syndrome among French-Canadian men.

Subjects' characteristics are shown in Table 1. The molecular screening of the *11β-HSD1* gene revealed the presence of four variants (Table 2). Three of these variants were located in intronic regions (g.4478T>G, g.10733G>C, and g.4437-4438insA). One sequence variant was identified in exon 6. Based on the predicted amino acid sequence, this polymorphism is silent and does not alter the glycine at codon 248 (c.744G>C or G248G). Screening of the promoter region did not reveal any sequence variations. The relative allele frequency of these variants is shown in Table 2. Intronic variants identified were tested for linkage disequilibrium. The two mutations in intron 3 (g.4478T>G and g.4437-4438insA) were in perfect linkage disequilibrium ($r^2 = 1.0$, $p < 0.0001$). Moreover, the single-nucleotide polymorphism (SNP) in intron 4 (g.10733G>C) was also in linkage disequilibrium with the SNP and the insertion in intron 3 ($r^2 = 0.83$, $p < 0.0001$ for both).

We then verified whether these variants were associated with components of the metabolic syndrome. Due to the very low frequency of the c.744G>C SNP (0.005 for 744C allele), association studies were not pursued further with this variant. Indices of obesity and plasma insulin and glucose levels in the fasting state and in response to an oral glucose tolerance test were not associated with any of the genetic variations identified (Table 3). For plasma lipoprotein/lipid levels, a significant difference was observed in plasma apolipoprotein B (apo B) levels. Indeed, carriers of the 10733C allele had plasma apo B levels of 1.13 ± 0.19, whereas G10733/G10733 homozygotes had plasma apo B levels of 1.07 ± 0.23 ($p = 0.04$). Similar results were observed for carriers of the 4478G and 4438insA alleles ($p = 0.06$ for both). This was the only association observed. Similar results were observed after adjustment for the effect of age, age and BMI, or age and visceral adipose tissue accumulation assessed by computed tomography (data not shown). We subsequently compared the frequency of the rare allele of each variant in subjects with and without the metabolic syndrome using criteria defined by the NCEP-ATPIII (1). The frequency of each rare allele (10733C, 4437-4438insA, and 4478G) in men with fewer than three components of the metabolic syndrome was similar to that in men with three or more components of the metabolic syndrome ($\chi^2 = 0.01$, $p = 0.92$; $\chi^2 = 0.01$, $p = 0.91$; $\chi^2 = 0.01$, $p = 0.91$, respectively).

Because the gene encoding the *11β-HSD1* has been shown to be involved in abdominal obesity and its related complications, it was relevant to search for molecular variations that might have an impact on features of the metabolic syndrome. The present study addressed this question, and we report the identification of four *11β-HSD1* variants. Two of these sequence variants were SNPs in intronic regions of the *11β-HSD1* gene (g.4478T>G and g.10733G>C). One adenine insertion (g.4437-4438insA) was identified in intron 3. The present study also reports one sequence variation in exon 6 that results in a nucleotide substitution (c.744G>C). However, this variant does not alter the amino acid sequence. The allele frequency of each

Table 2. Relative allele frequency of variants identified in the *11β-HSD1* gene

Sequence variant	Frequency of the rare allele
Intron 3 g.4478T>G	0.14
Intron 3 g.4437–4438insA	0.28
Intron 4 g.10733G>C	0.21
Exon 6 c.744G>C	0.005

Table 3. Subjects' characteristics according to each genotype

	4478T>G		4437–4438insA		10733G>C	
Variables	**4478 T/T**	**4478 G carriers**	**Wild type (w/w)**	**4437–4438insA carriers**	**10733 G/G**	**10733 C carriers**
BMI (kg/m^2)	29.5 ± 4.3 (139)	29.6 ± 4.2 (78)	29.5 ± 4.3 (139)	29.6 ± 4.2 (78)	29.5 ± 4.4 (129)	29.7 ± 4.2 (88)
Waist circumference (cm)	101.3 ± 11.6 (138)	101.6 ± 11.0 (78)	101.3 ± 11.6 (138)	101.6 ± 11.0 (78)	101.0 ± 11.6 (128)	102.0 ± 11.1 (88)
VAT accumulation (cm^3)	164.7 ± 63.0 (138)	176.1 ± 65.0 (76)	164.7 ± 63.0 (138)	176.1 ± 65.0 (76)	161.95 ± 59.89 (128)	178.87 ± 68.30 (86)
High-density lipoprotein-cholesterol (mM)	0.93 ± 0.22 (138)	0.90 ± 0.17 (76)	0.93 ± 0.22 (138)	0.90 ± 0.17 (76)	0.94 ± 0.21 (128)	0.89 ± 0.20 (86)
Triglycerides (mM)	2.19 ± 1.10 (138)	2.29 ± 1.11 (76)	2.19 ± 1.10 (138)	2.29 ± 1.11 (76)	2.15 ± 1.09 (128)	2.34 ± 1.13 (86)
Total apo B (g/L)	1.07 ± 0.23 (136)	1.13 ± 0.19 (75)	1.07 ± 0.23 (136)	1.13 ± 0.19 (75)	1.07 ± 0.23 (126)	1.13 ± 0.19 (85)*
Systolic blood pressure (mm Hg)	121.9 ± 9.6 (130)	123.1 ± 9.6 (70)	121.9 ± 9.6 (130)	123.1 ± 9.6 (70)	121.7 ± 9.9 (120)	123.2 ± 9.1 (80)
Diastolic blood pressure (mm Hg)	82.2 ± 7.5 (130)	81.5 ± 6.7 (70)	82.2 ± 7.5 (130)	81.5 ± 6.7 (70)	82.1 ± 7.6 (120)	81.7 ± 6.7 (80)
Fasting glucose (mM)	5.47 ± 0.56 (138)	5.51 ± 0.59 (78)	5.47 ± 0.56 (138)	5.51 ± 0.59 (78)	5.46 ± 0.55 (128)	5.51 ± 0.60 (88)
Fasting insulin (pM)	104.3 ± 78.9 (138)	102.6 ± 64.3 (78)	104.3 ± 78.9 (138)	102.6 ± 64.3 (78)	99.6 ± 63.9 (128)	109.6 ± 86.3 (88)
HOMA IR	3.64 ± 2.98 (138)	3.56 ± 2.63 (78)	3.64 ± 2.98 (138)	3.56 ± 2.63 (78)	3.49 ± 2.65 (128)	3.79 ± 3.14 (88)

HOMA-IR, homeostasis model assessment of insulin resistance; VAT, visceral adipose tissue. Results are expressed as means ± SD. Number of subjects is shown in parentheses.
* Significantly different from wild-type homozygotes ($p < 0.05$).

polymorphism was determined in a sample of French-Canadian men. The genotype frequency for all variants identified did not deviate from Hardy-Weinberg predictions. To ascertain the contribution of this gene to the development of the metabolic syndrome, we tested linkage disequilibrium of the three intronic variants and performed association studies. The three SNPs were in linkage disequilibrium. Further analyses did not reveal any association among these mutations and components of the metabolic syndrome as defined by the NCEP-ATPIII, suggesting that these variants are unlikely to be the molecular defect explaining the observed phenotype. Among all variables tested, apo B level was the only one to be significantly different between genotype groups. Power analyses revealed that at an α-level of 0.05, plasma apo B was the variable with the highest power to detect this association. At this point, we cannot exclude the possibility that the lack of association with other variables is the reflection of a low power rather than an absence of association.

The insertion in intron 3 has been previously reported by Gelernter-Yaniv et al. (6). They observed in a study sample of healthy overweight and normal weight children from different racial backgrounds that this variant was associated with greater body mass and with altered body composition and insulin resistance. Differences in the genetic background of these populations may explain inconsistencies observed between the present study and the study by Gelernter-Yaniv et al. As mentioned by the authors, it is also possible that this association may be due to linkage disequilibrium with another mutation in a gene located in the vicinity of the *11β-HSD1* gene, which is not found in the French-Canadian population. In addition, the lack of association might be attributable to the low number of subjects on which the present study is based, although Gelernter-Yaniv et al. have reported a positive association in a relatively similar sample size (263 vs. 217) (6). Caramelli et al. have also identified two variants in 12 obese and nonobese Italian women. One of them is the insertion in intron 3, and the second is an 11-bp deletion in intron 1 (7). These variants were found in either abdominally obese subjects or control subjects, suggesting that these molecular defects were not involved in the etiology of visceral obesity. In the present study, we did not observe the deletion in intron 1, suggesting that this variant may be specific to the Italian population. The *11β-HSD1* gene has been screened previously in a woman characterized by central obesity and other hormonal dysfunctions (8). The authors were not able to associate genetic variations in the *11β-HSD1* gene with these metabolic alterations. Both variants identified in intron 3 have been previously described in individuals with cortisone reductase deficiency (9). Transcriptional activity assays have demonstrated 2.5 times lower *11β-HSD1* activity for the two mutated *HSD11B1* constructs. However, the authors suggested that these mutations were not the molecular defect explaining the cortisone reductase deficiency because 25% of unaffected controls were heterozygous, and 3% were homozygous for these polymorphisms. They also identified two mutations in the hexose-6-phosphate dehydrogenase gene (H6PD 620ins29bp621 and H6PD R453Q) and showed epistasis with variants identified in the *11β-HSD1* gene to cause the cortisone reductase deficiency. At this point, we cannot exclude the possibility that mutations in the hexose-6-phosphate dehydrogenase gene might interact with variants identified in *11β-HSD1* to influence the development of the metabolic syndrome.

In conclusion, results obtained in the present study come at a time of great interest in the role of *11β-HSD1* in central obesity and its complications. This study did not reveal the presence of genetic variants in the *11β-HSD1* gene that could help understand the impact of local excess glucocorticoids in the metabolic syndrome among French-Canadian men. However, further studies involving *11β-HSD1* activity measurements and cortisol/cortisone metabolites are clearly needed to confirm results of the present study. In addition, these data do not exclude the possibility that genetic variations in the *11β-HSD1* gene may be associated with susceptibility to abdominal obesity and related metabolic complications in other populations. The identification of additional genetic variants in the *11β-HSD1* gene provides helpful tools for further investigations of association with the metabolic syndrome to confirm these results.

Research Methods and Procedures

Subjects

DNA was obtained from a cohort of 217 French-Canadian men who were recruited through the media in the greater Quebec City area and who were selected to cover a wide range of body fatness values (10). We searched for genetic variations in genomic DNA from 36 men with the metabolic syndrome, as defined by the NCEP-ATPIII, and two controls. The genotype/allele frequencies of the newly identified mutations were assessed in the cohort of 217 French-Canadian men. Subjects gave their written consent to participate in this study, which received the approval of the Laval University Ethics Committee.

Methods

The exons, the exon-intron splicing boundaries, and the 5′ and 3′ regions of the human *11β-HSD1* gene were sequenced to screen for DNA variants. Table 4 shows previously described primers used to amplify each fragment (7). Primers of the promoter region were designed using the Primer 3.0 software available on the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research server (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Polymerase chain reaction (PCR) conditions were as follow: reaction volume was 50 μmL, 1 unit

Table 4. PCR primers for genomic amplification of *11β-HSD1* promoter and exons

Gene region	Primer sequences*	Annealing temperature	Product size (bp)
Promoter region	F-attgaacttgagtgtggggc	68 °C	721
	R-acgcatgggtacttacctgg		
Exon 1	F-tgcctgagactactccagcct	62 °C	264
	R-tcctccaaaatgagaagcatgg		
Exon 2	F-ccaacttgggtatggtcctcacttc	59 °C	214
	R-acacatctgagcatgtgacggtacac		
Exons 3–4	F-tgagcaatctctcatttaagccc	59 °C	539
	R-tgtccctgtcccacttaccagcc		
Exon 5	F-atgggcagccttattaaccca	59 °C	245
	R-catgtaccctagaacttaaagta		
Exon 6	F-tagactgtcctagtcagataac	63 °C	369
	R-tctggggacactctcggaag		

* All but promoter primers were previously published (7).

of AmpliTaqDNA polymerase (PerkinElmer Life and Analytical Sciences, Boston, MA), 5 μL of 10× PCR buffer recommended by the manufacturer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 8.4 μL of each primer at a final concentration of 7.5 μM, and 0.1 μg of genomic DNA. The annealing temperature for each fragment is shown in Table 4. Sequencing reactions were performed using BigDyeTerminator version 3.0 cycle sequencing (ABI Prism; PE Applied Biosystems, Foster City, CA), and the products were analyzed on an ABI 3100 automated DNA sequencer (PE Applied Biosystem). The gel files were processed using the ABI Prism3100 data collection software applied biosystem version 1.1 and ABI Prism DNA sequencing analysis software (PE Applied Biosystems), then assembled and analyzed using the STADEN preGap4 and Gap4 programs.

Newly identified SNPs were genotyped using PCR-restriction fragment-length polymorphism-based methods. Primers were designed using sequences available on GenBank (accession nos. AY044083 and AY044084) and the Primer 3.0 software available on the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research server (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

The SNP in intron 3 (g.4478T>G) was genotyped using the primers F-CAA GAG CTT TTG GGA GGA GA and R-TTG CGC ACA TGG TGA ATA TC in conditions described above. Annealing temperature was 65 °C, and PCR products were digested with the restriction enzyme MnlI, electrophoresed through a 20% acrylamide gel, and stained with ethidium bromide.

The SNP in intron 4 (g.10733G>C) was genotyped using the primers F-CCA CCC TAT GCC TTC GAT AC and R-ACA CTT GGA CAC AGG AAG GG, and PCR conditions were as described above. Annealing temperature was 66 °C. After amplification, PCR products were digested with HphI and electrophoresed through an 8% acrylamide gel stained in ethidium bromide.

The variant in exon 6 (c.744G>C) was genotyped using the primers for exon 6 shown in Table 4. PCR products were digested with the restriction enzyme AciI, electrophoresed through a 8% acrylamide gel, and stained with ethidium bromide.

Finally, the insertion in intron 3 (g.4437-4438insA) was genotyped by amplification using the primers for exons 3 and 4 shown in Table 4 followed by direct sequencing of genomic DNA.

Statistical Analyses

Genotype and allele frequencies were determined with the gene counting method. Significant deviation from Hardy-Weinberg equilibrium was tested by χ^2. Linkage disequilibrium was tested using "SNPAlyze" software (DYNACOM Co., Ltd., Japan). Analyses were performed with SAS (SAS Institute, Cary, NC). Power analyses were performed with JMP version 3.1.6.2 from SAS Institute. $p < 0.05$ was used to identify a statistically significant difference.

Acknowledgments

This study was supported by Grant MOP-44074 from the Canadian Institutes of Health Research (CIHR) and by the Heart and Stroke Foundation of Canada. J.R. received a fellowship from the CIHR. C.B. is the recipient of a stu-

dentship from the Fonds de la Recherche en Santé du Québec. J.P.D. holds the Chair of Human Nutrition, Lipidology, and Prevention of Cardiovascular Disease supported by Provigo and Parke-Davis/Warner-Lambert, Canada. A.T. and M.C.V. are research scholars from the CIHR and the Fonds de la Recherche en Santé du Québec, respectively.

References

1. **Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.** Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–97.
2. **Bouchard C.** Genetics and the metabolic syndrome. *Int J Obes.* 1995;19(Suppl 1):S52–9.
3. **Bujalska IJ, Kumar S, Stewart PM.** Does central obesity reflect "Cushing's disease of the omentum"? *Lancet*. 1997; 349:1210–3.
4. **Wajchenberg BL, Bosco A, Marone MM, et al.** Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *J Clin Endocrinol Metab*. 1995;80:2791–4.
5. **Masuzaki H, Paterson J, Shinyama H, et al.** A transgenic model of visceral obesity and the metabolic syndrome. *Science*. 2001;294:2166–70.
6. **Gelernter-Yaniv L, Feng N, Sebring NG, Hochberg Z, Yanovski JA.** Associations between a polymorphism in the 11 beta hydroxysteroid dehydrogenase type I gene and body composition. *Int J Obes Relat Metab Disord*. 2003; 27:983–6.
7. **Caramelli E, Strippoli P, Di Giacomi T, Tietz C, Carinci P, Pasquali R.** Lack of mutations of type 1 11beta-hydroxysteroid dehydrogenase gene in patients with abdominal obesity. *Endocr Res*. 2001;27:47–61.
8. **Jamieson A, Wallace AM, Andrew R, et al.** Apparent cortisone reductase deficiency: a functional defect in 11beta-hydroxysteroid dehydrogenase type 1. *J Clin Endocrinol Metab*. 1999;84:3570–4.
9. **Draper N, Walker EA, Bujalska IJ, et al.** Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet*. 2003;34: 434–9.
10. **Berthier MT, Couillard C, Prud'homme D, et al.** Effects of the FABP2 A54T mutation on triglyceride metabolism of viscerally obese men. *Obes Res.* 2001;9:668–75.