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Research

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Impact of *NMT1* Gene Polymorphisms on Features of the Metabolic Syndrome among Severely Obese Patients

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ABSTRACT

Introduction: N-myristoyltransferase (NMT) is implicated in myristoylation, required for biological activities of several proteins. Its gene N-myristoyltransferase 1 (*NMT1*) has been found to be overexpressed and hypermethylated in Visceral Adipose Tissue (VAT) of severely obese individuals with Metabolic Syndrome (MetS+) *versus* without (MetS-).
Objective: The aim of this study was to verify the associations between *NMT1* gene polymorphisms Single Nucleotide Polymorphisms (SNPs) and metabolic complications among obese subjects.
Methods: Associations between SNPs and determinants of MetS were tested with 1752 obese participants undergoing a bariatric surgery. The effect of selected SNPs on methylation, and correlation with expression levels of *NMT1* were verified in subgroups.
Results: Rs2239921 was significantly associated with systolic ($p=0.03$) and diastolic ($p<0.0001$) blood pressures. Rs2239923 was associated with plasma High Density Lipoprotein-Cholesterol or HDL-Cholesterol (HDL-C) levels ($p=0.05$), while rs2269746 was associated with Low Density Lipoprotein-Cholesterol or LDL-Cholesterol (LDL-C) ($p=0.006$) and Total-Cholesterol (Total-C) levels ($p=0.004$). Rs1005136 ($p=0.03$), rs8066395 ($p=0.03$) or rs2157840 ($p=0.04$) were associated with plasma concentrations of C-Reactive Protein (CRP). Phenotype-associated SNPs were associated with *NMT1* methylation levels of six CpG sites. *NMT1* methylation levels of one CpG site, cg10755730, correlated with gene expression levels ($r=0.57$; $p=0.04$).
Conclusion: These results suggest that the presence of *NMT1* SNPs is associated with altered plasma lipid levels as well as with increased inflammation markers and blood pressure among severely obese patients.

KEYWORDS: Obesity; Epigenetics; Genomics; Lipids; Insulin; Myristoylation.

ABBREVIATIONS: BMI: Body Mass Index; CRP: C-Reactive Protein; CVD: Cardiovascular disease; DBP: Diastolic Blood Pressure; HDL-C: High Density Lipoprotein-Cholesterol; HMZ: Homozygote; HTZ: Heterozygote; HWE: Hardy-Weinberg Equilibrium; LDL-C: Low Density Lipoprotein-Cholesterol; MetS: Metabolic Syndrome; *NMT1*: N-myristoyltransferase 1; SBP: Systolic Blood Pressure; SNP: Single Nucleotide Polymorphism; TG: Triglycerides; T2D: Type 2 Diabetes; VAT: Visceral Adipose Tissue; DNA: Deoxyribonucleic acid; NCEP-ATPIII: National Cholesterol Education Program Adult Treatment Panel III.

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INTRODUCTION

Obesity is a major health problem worldwide due to the imbalance created by physical inactivity and the abundance of food, which are influenced by intrinsic and extrinsic factors.[1] This condition brings about significantly increased risk of Cardiovascular diseases (CVD) and Type 2 Diabetes (T2D)[2] through a constellation of risk factors characterizing the metabolic syndrome (MetS).[3] However, nearly 20% of severely obese individuals remain exempt of MetS.[3] This could be partly explained by genetic factors and epigenetic modifications. Epigenetics modulate gene expression and can also be involved in the process leading to increased comorbidities in certain obese individuals, as they are both transmissible and influenced by the environment.[4] It encompasses strong mechanisms that regulate gene expression without affecting the Deoxyribonucleic acid (DNA) sequence.[5] Also, epigenetic marks, such as CpG dinucleotides, are more frequent in promoter regions and first exons of specific genes,[6] consistent with a major role for methylation in the regulation of gene expression. We have previously shown that methylation levels are associated with MetS[7] and its features.[8]

The *NMT1* gene, located on chromosome 17, encodes the enzyme N-myristoyltransferase 1 (*NMT1*). It catalyzes the irreversible reaction of myristoylation,[9] which makes a specific covalent linkage between myristic acid, a 14-carbon saturated fatty acid, and the NH2-terminal glycine of a protein.[10] For many proteins, myristoylation is essential for stability and functions, such as protein-protein interactions, membrane attachment and cellular localization.[11] Myristoylation may also be involved in the regulation of gene transcription through modification of DNA-binding proteins.[11] *NMT1* has not been previously associated with MetS, but as it induces important protein modifications,[12] and is largely linked with disease state,[13] it may potentially be related to obesity comorbidities. Previous work by our team observed *NMT1* as being differentially expressed and methylated between subjects with MetS (Mets+) and without MetS (MetS-).[14,15] The aim of the current study was thus to test for specific associations between *NMT1* genetic variations and metabolic complications among severely obese patients. Afterwards, the association between the phenotype-associated SNPs and methylation sites, as well as between these sites and expression levels, were tested to further understand the potential underlying mechanisms relating *NMT1* to obesity-related complications. We therefore, hypothesize that genetic variations/SNPs in *NMT1* are associated with features of MetS and that *NMT1* gene methylation levels are associated with obesity-related metabolic complications, based on the role of methylation in the regulation of gene expression.[16]

MATERIAL AND METHODS

Subjects Selection

A total of 1752 severely obese men (N=545) and women (N=1207) were recruited among patients undergoing a bariatric surgery (biliopancreatic diversion with duodenal switch) since June 2000 at the Québec Heart and Lung Institute (Québec City, Québec, Canada). The surgical protocol has been described elsewhere.[17] Body weight, height, waist girth and resting Systolic Blood Pressure (SBP) and Diastolic Blood Pressures (DBP) were measured before the surgery using standardized procedures. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. C-reactive protein (CRP) concentrations in plasma were measured with an high-sensitivity C-reactive protein (hs-CRP) immunoassay using a monoclonal antibody coated with polystyrene particles.[18] The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) diagnosis criteria[19] were used to determine the presence of MetS. All subjects included in the study provided a written informed consent and Université Laval ethics committee approved the study. Tissue specimens were obtained from the Biobank of the Institut universitaire de cardiologie et de pneumologie de Québec according to institutionally-approved management modalities.

Genotyping

Genomic DNA was extracted from the blood buffy coat using the GenElute Blood Genomic DNA kit (Sigma, St Louis, MO, USA). Based on differential expression and methylation of the *NMT1* gene in a previous dataset,[14] 11 tag Single Nucleotide Polymorphisms (tSNPs) spanning promoter (2 kb), coding and intronic regions of the *NMT1* gene in addition to the 3' gene region (2 kb) were selected for analysis using the Tagger selection algorithm of the Haploview software (pairwise tagging, R2 ≥0.80).[20] This strategy allowed covering 100% of the genetic variability of the common polymorphisms (MAF≥1%) at the *NMT1* locus in the Caucasian population (CEU HapMap). In addition, rs2157840 SNP was included in this study due to its close localization to the cg00693004 CpG site previously found to be differentially methylated between MetS+ and MetS- subjects.[17] We thus ended up with a set of 12 SNPs analyzed in the current study. SNPs were genotyped, using the QuantStudio™ 12K Flex OpenArray® AccuFill™ system (Applied Biosystems) and analyzed with TaqMan Genotyper v1.3 (Life Technologies).

DNA Methylation Analysis

A second subgroup of 32 obese individuals chosen among the larger group was used for DNA methylation analysis, including the 14 subjects (MetS+, N=7; MetS-, N=7) selected for gene expression profiling.[14] The 18 obese individuals (MetS+, N=9; MetS-, N=9) added to the 14 initially studied[15] were selected to fulfil initial selection criteria[14] and to represent extremes of the MetS diagnosis criteria spectrum. Genomic DNA extraction was achieved from 200 mg of visceral adipose tissue (VAT) using the DNeasy Blood and Tissue kit (QIAGEN, Mississauga, Ontario, Canada). Bisulfite conversion was conducted on 1 µg of DNA, and quantitative DNA methylation analysis was carried out at the McGill University and Génome Québec Innovation

Centre (Montreal, Canada). Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA) were processed according to the manufacturer's instructions. The BeadChips interrogate more than 485000 methylation sites at single-nucleotide resolution. Methylation data were visualized and analyzed with the GenomeStudio software version 2011.1 (Illumina Inc.) and the methylation module. Methylation levels (beta values; β) were estimated as the ratio of signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals of the alleles (β value= C/(T+C)). We applied internal control probe pairs for data correction (background subtraction and normalization). CpG sites located within the *NMT1* locus and promoter region were extracted using the GenomeStudio Methylation Module, thus leading to a total of 26 CpG sites analyzed in this study. *NMT1* was identified as differentially methylated [(Diff score=341.86) based on the differentially methylated CpG site cg00693004 (chr17:43151433, according to genome build 37).

Gene Expression

Expression data for the *NMT1* gene presented here were retrieved from a previous study aimed at the identification of differentially methylated genes between MetS+ and MetS- severely obese men. The protocol leading to the identification of *NMT1* as being differentially expressed between severely obese (BMI >40 kg/m^2) MetS+ and MetS- men has been previously described.[14] Briefly, 14 severely obese men with and without MetS, who did not take any medication to treat MetS components, were matched as closely as possible for age, BMI and smoking status. Characteristics of individuals sorted by MetS groups are available elsewhere.[14] Even if it is not listed in the tables due to the large number of genes discovered, this investigation led to the determination of *NMT1* gene as being overexpressed within VAT of the MetS+ *versus* MetS- subsets (1.35-fold; p=0.04).

Statistical Analysis

At first, phenotypic differences between the MetS+ and MetS- groups were tested for the entire cohort. Gene expression microarray analysis was conducted using unpaired Student's t-test, and differentially expressed genes were compared between MetS+ and MetS- groups. Hardy-Weinberg Equilibrium (HWE) was verified. For SNPs showing a frequency of rare homozygotes below 5%, homozygotes for the rare allele were merged to heterozygotes for statistical analysis (6 SNPs: rs41484746, rs8066395, rs10491142, rs2239921, rs2239922 and rs2269746). The Generalized Linear Model (GLM) procedure was used to test associations between SNPs and MetS components, with adjustments for age, sex, BMI and medication to treat MetS features when appropriate. When a significant SNP effect was identified, all pairwise comparisons among genotype groups were performed using least square means and Student's t-tests. Pearson correlation co-efficients were computed to assess the relationship between methylation, expression and MetS components. P-values were calculated for all associations and were considered to be statistically significant if the P-value was less than 0.05. Statistical analysis were performed with Statistical Analysis Software (SAS) software version 9.3. Phenotypic data are presented as mean±SD.

RESULTS

Cohort Description

From the study sample of 1752 severely obese subjects, 1745 patients were classified as being MetS+ (n=1428) or MetS- (n=317 or 20.19%). All characteristics shown in Table 1 were significantly different between MetS+ and MetS- groups, except BMI and plasma CRP levels. The MetS+ group had a higher mean age and showed significantly higher values for waist girth, fasting glucose, Triglycerides (TG), total-C/HDL-C ratio, SBP and DBP. The MetS- group showed significantly higher plasma concentrations for total-C, LDL-C and HDL-C.

Identification of *NMT1* SNPs

Regarding the 12 SNPs, 8 were intronic and 4 were exonic, 2 of them being in the 3' untranslated region (exon 12) (Table 2). Exonic SNPs rs2239922 and rs2239923 were synonymous variations. These 12 tSNPs were further genotyped in the whole cohort of 1752 participants. Genotype distribution and HWE p-values are also shown in Table 2.

Association of *NMT1* SNPs with features of the MetS

Associations between tSNPs, and plasma fasting glucose, lipid, CRP levels as well as blood pressure, taking into account the confounding effects of age, sex, BMI and medication, were tested. Significant associations were observed for rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136 (Table 3). Carriers of the rare allele for rs2239921 showed lower systolic (p=0.03) and diastolic (p<0.0001) blood pressures than wild-type homozygotes. Rare homozygotes for rs2239923 displayed elevated HDL-C levels compared to the other genotype groups (p=0.05), while carriers of the wild-type genotype for rs2269746 demonstrated higher levels of LDL (p=0.006) and total-C (p=0.004) than carriers of the rare allele. Homozygotes of the wild-type allele for rs1005136, rs8066395 and rs2157840 displayed elevated plasma CRP levels (p=0.03, p=0.03 and p=0.04 respectively). Two trends were also found, the first for association between rs12449933 with elevated plasma TG concentrations (p=0.08; 2.15±1.58 *vs.* 1.82±1.08 *vs.* 1.78±0.94 mmol/L) in rare homozygotes, and the second in carriers of the wild-type genotype for rs41484746 with higher BMI (p=0.06; 51.8±8.7 *vs.* 50.8±8.2 kg/m^2) and waist girth (p=0.06; 140.8±17.9 *vs.* 139.1±16.8) than carriers of the rare allele.

Gene Methylation and Expression Analysis

With the perspective of trying to better understand the mechanism underlying the association between *NMT1*'s SNPs

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	All	MetS+	MetS-	P value
Number of Subjects (% male)	1752(31.1)	1428(33.61)	317(20.19)	—
Age (years)	43.0±10.6	44.0±10.6	38.7±9.8	<0.0001
BMI (kg/m^2)	51.8±8.9	52.0±9.0	51.2±8.7	0.32
Waist girth (cm)	140.6±17.7	141.7±17.4	135.5±18.5	<0.0001
CRP (mg/l)	11.04±9.12	11.22±9.16	10.42±9.00	0.05
Fasting glucose (mmol/L)	6.53±2.32	6.85±2.41	5.11±0.98	<0.0001
Lipid profile (mmol/L)				
TG	1.83±1.08	1.98±1.12	1.17±0.37	<0.0001
Total-C	4.70±0.95	4.68±0.98	4.80±0.82	0.05
LDL-C	2.67±0.83	2.65±0.84	2.80±0.75	0.02
HDL-C	1.24±0.35	1.18±0.32	1.49±0.37	<0.0001
Total-C/HDL-C	4.03±1.29	4.17±1.34	3.33±0.74	<0.0001
Blood Pressure (mm Hg)				
SBP	139.0±17.1	140.0±17.3	134.6±15.6	0.0006
DBP	83.8±11.5	84.0±11.7	83.1±10.3	0.04

Values are presented as mean±SD.
Abbreviations: MetS: Metabolic Syndrome; BMI: Body Mass Index; CRP: C-Reactive Protein; TG: triglycerides; Total-C: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SD: Standard Deviation.

Table 1: Subjects characteristics.

SNPs	Number of genotypes	Common HMZ (WT)	HTZ	Rare HMZ	Localization[a]	Other designation[b]	Region	MAF	HWE P values
rs12449933	1737	1060	587	90	chr17:43137201	c.-1497C>T	Intron 1	0.22	0.46
rs41484746	1740	1458	268	14	chr17:43142664	c.131+3836A>G	Intron 1	0.09	0.66
rs8066395	1716	1108	547	61	chr17:43147582	c.131+8754A>G	Intron 1	0.19	0.52
rs2157839	1734	772	765	197	chr17:43151400	c.132-7612T>C	Intron 1	0.33	0.72
rs2157840	1731	530	843	358	chr17:43151473	c.132-7539G>T	Intron 1	0.45	0.50
rs1005136	1725	550	834	341	chr17:43164246	c.385+226G>T	Intron 3	0.44	0.44
rs10491142	1738	1308	400	30	chr17:43167863	c.386-3190G>C	Intron 3	0.13	0.93
rs2239921	1735	1585	149	1	chr17:43170907	c.386-146C>T	Intron 3	0.04	0.19
rs2239922	1740	1492	234	14	chr17:43175906	c.870C>T	Exon 7	0.08	0.15
rs2239923	1733	850	728	155	chr17:43176804	c.916C>T	Exon 8	0.30	0.96
rs1053733	1730	585	829	316	chr17:43183028	c.*21G>A	Exon 12 (3'-UTR)	0.42	0.46
rs2269746	1740	1535	198	7	chr17:43185023	c.*2016C>A	Exon 12 (3'-UTR)	0.06	0.82

[a]Position according to genome build 37. [b]Reference sequence : NM_021079.
Abbreviations: SNP: Single Nucleotide Polymorphism; HMZ: Homozygote; WT: Wild-type; HTZ: Heterozygote; MAF: Minor Allele Frequency; HWE: Hardy-Weinberg Equilibrium.

Table 2: Genotype distribution and localization of selected *NMT1* SNPs.

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Phenotypes	rs12449933 Means[a] Common HMZ (WT)	HTZ	Rare HMZ	*P* values[b]	rs41484746 Means Common HMZ (WT)	Rare allele carrier	*P* values
Number of subjects (N)	1055	584	90	—	1450	281	—
BMI (kg/m^2)	51.8± 8.8	51.5±8.0	50.8±10.1	0.54	51.8±8.7	50.8±8.2	0.06
Waist girth (cm)	140.7±17.9	140.7±17.0	138.0±20.3	0.25	140.8±17.9	139.1±16.8	0.06
CRP Protein (mg/l)	10.88± 8.32	10.53±8.31	11.67±9.11	0.87	10.96±8.39	10.01±8.10	0.49
Fasting glucose (mmol/l)	6.53±2.33	6.52±2.29	6.85±2.47	0.42	6.53±2.33	6.61±2.33	0.45
Lipid profile (mmol/l)							
TG	1.82±1.08	1.78±0.94	2.15±1.58	0.08	1.83±1.06	1.82±1.09	0.22
Total-C	4.68±0.93	4.69±0.95	4.84±0.87	0.49	4.69±0.92	4.70±1.01	0.97
LDL-C	2.67±0.83	2.69±0.82	2.72±0.75	0.36	2.67±0.81	2.71±0.88	0.51
HDL-C	1.23±0.32	1.24±0.38	1.25±0.48	0.54	1.24±0.36	1.22±0.28	0.81
Total-C/HDL-C	4.01±1.22	4.03±1.42	4.19±1.30	0.27	4.02±1.32	4.01±1.10	0.90
Blood pressure (mmHg)							
SBP	138.9±16.6	138.8±16.7	137.5±17.8	0.33	138.7±16.6	139.4±17.3	0.56
DBP	83.8±11.5	83.9±10.7	82.6±11.3	0.41	83.9±11.3	82.96±10.8	0.30

Phenotypes	rs2239921 Means Common HMZ (WT)	Rare allele carrier	*P* values	rs2239923 Means Common HMZ (WT)	HTZ	Rare HMZ	*P* Values
Number of subjects (N)	1578	148	—	846	725	155	—
BMI (kg/m^2)	51.7±8.5	52.0±9.7	0.66	51.5±8.6	51.7±8.4	52.5±9.5	0.30
Waist girth (cm)	140.5±17.5	141.9±20.1	0.18	140.8±17.5	140.3±18.0	141.0±17.8	0.81
CRP Protein (mg/l)	10.72±8.25	11.83±9.47	0.73	10.64±8.42	10.96±8.38	11.57±7.93	0.27
Fasting glucose	6.56 ±2.35	6.31±2.04	0.69	6.52±2.27	6.53±2.30	6.62±2.56	0.87
Lipid profile (mmol/l)							
TG	1.81±1.00	1.93±1.64	0.45	1.82±1.06	1.82±1.09	1.80±1.02	0.85
Total-C	4.68±0.94	4.76±0.89	0.81	4.70±0.94	4.69±0.93	4.64±0.92	0.74
LDL-C	2.67±0.82	2.74±0.81	0.93	2.69±0.82	2.67±0.83	2.59±0.79	0.96
HDL-C	1.24±0.36	1.21±0.29	0.71	1.24±0.37	1.23±0.35	1.26±0.27	0.05
Total-C/HDL-C	4.01±1.30	4.15±1.23	0.47	4.06±1.43	4.02±1.16	3.82±1.02	0.40
Blood pressure (mmHg)							
SBP	138.9±16.7	138.1±17.6	0.03	139.3±17.3	138.7±16.6	136.9±13.9	0.82
DBP	83.9±11.1	81.2±11.5	<0.0001	83.9±11.4	83.4±11.1	84.2±10.5	0.34

Phenotypes	rs2269746 Means			rs8066395 Means		
	Common HMZ (WT)	Rare allele carrier	*P* values	Common HMZ (WT)	Rare allele carrier	*P* values
Number of subjects (N)	1527	204	—	1102	605	—
BMI (kg/m^2)	51.6±8.5	52.3±9.2	0.39	51.8±8.9	51.5±8.0	0.50
Waist girth (cm)	140.5±17.7	140.9±18.0	0.88	140.4±18.0	140.8±17.2	0.46
CRP Protein (mg/l)	10.79±8.34	10.89±8.48	0.29	11.21±8.52	10.03±7.99	0.03
Fasting glucose	6.56±2.34	6.40±2.22	0.93	6.61±2.39	6.40±2.20	0.26
Lipid profile (mmol/l)						
TG	1.83±1.10	1.77±0.83	0.97	1.85±1.15	1.78±0.91	0.58
Total-C	4.71±0.94	4.56±0.89	0.004	4.69±0.93	4.71±0.94	0.70
LDL-C	2.69±0.82	2.56±0.81	0.006	2.67±0.82	2.70±0.83	0.93
HDL-C	1.24±0.36	1.21±0.32	0.61	1.23±0.34	1.25±0.37	0.84
Total-C/HDL-C	4.03±1.29	3.99±1.32	0.18	4.05±1.33	3.99±1.23	0.64
Blood pressure (mmHg)						
SBP	138.7±16.7	140.1±17.4	0.12	138.6±16.7	139.2±16.8	0.61
DBP	83.6±11.1	84.8±11.7	0.42	83.7±11.1	83.9±11.3	0.78

Phenotypes	rs2157840 Means				rs1005136 Means			
	Common HMZ (WT)	HTZ	Rare HMZ	*P* values	Common HMZ (WT)	HTZ	Rare HMZ	*P* values
Number of subjects (N)	527	838	357	—	547	829	340	—
BMI (kg/m^2)	52.2±9.2	51.5±8.2	51.5±8.6	0.26	52.1±9.2	51.5±8.2	51.5±8.7	0.23
Waist girth (cm)	140.9±17.8	140.1±17.6	141.2±17.8	0.63	140.6±17.8	140.2±17.8	141.3±17.7	0.59
CRP Protein (mg/l)	11.78±8.59	10.36±8.29	10.57±8.13	0.04	11.81±8.60	10.21±8.23	10.63±8.19	0.03
Fasting glucose	6.55±2.30	6.54±2.38	6.50±2.22	0.72	6.53±2.31	6.55±2.38	6.47±2.12	0.43
Lipid profile (mmol/l)								
TG	1.86±1.25	1.79±0.95	1.86±1.06	0.78	1.86±1.25	1.79±0.96	1.86±1.02	0.85
Total-C	4.67±0.93	4.69±0.95	4.73±0.93	0.98	4.67±0.94	4.69±0.93	4.75±0.94	0.86
LDL-C	2.64±0.81	2.68±0.84	2.72±0.80	0.74	2.64±0.82	2.68±0.83	2.73±0.80	0.43
HDL-C	1.23±0.29	1.23±0.35	1.25±0.44	0.38	1.23±0.29	1.23±0.35	1.26±0.44	0.68
Total-C/HDL-C	3.98±1.11	4.03±1.32	4.08±1.46	0.77	3.99±1.17	4.02±1.30	4.09±1.47	0.71
Blood pressure (mmHg)								
SBP	139.0±16.4	138.5±17.0	139.5±16.5	0.92	138.8±16.3	138.7±17.2	139.0±16.3	0.99
DBP	84.1±11.0	83.4±11.3	84.0±11.3	0.67	83.9±11.0	83.5±11.2	83.9±11.4	0.68

[a]Values presented (means±SD) are untransformed and unadjusted. [b]*P* values obtained are adjusted for the effect of age, sex, BMI and medication use except for BMI and waist girth which were adjusted for age and sex.
Abbreviations: HMZ: Homozygote; WT: Wild-type; HTZ: Heterozygote; N: Number; BMI: Body Mass Index; CRP: C-Reactive Protein; TG: Triglycerides; Total-C: Total cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SD: Standard Deviation.

Table 3: Significant genotype differences identified between *NMT1* SNPs, subjects' characteristics, CRP, fasting glucose, lipid profile and blood pressure.

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and CVD risk factors, we investigated gene methylation and expression levels. Between genotype group differences in methylation and expression levels were tested only for CVD risk factor-associated SNPs (rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136). Methylation levels were available for 26 CpG sites distributed within or near the gene; the precise locations are presented in Table 4. Six CpG sites were significantly associated with phenotype-associated SNPs. There was an association between methylation and gene expression levels only for cg10755730 (r=0.574; p=0.04; Table 5).

DISCUSSION

Only a few studies on *NMT1* are related to obesity or T2D[21] with most studies related to cancer.[22] The tyrosine-kinases c-Src family, which includes important oncogenesis-related molecules,[23] was identified among its main substrates.[24] For this reason, it represents a therapeutic target for cancer treatment,[22] and has drawn a lot of attention recently. Moreover, known *NMT1* substrates are kinases, phosphatases and G-proteins,[21] involved in numerous signal-transduction cascades.[10] *NMT1* is thus implicated in several metabolic processes, from cell development to apoptosis.[25] In a transcriptomic experiment, we observed that the *NMT1* gene was overexpressed in VAT of MetS+ obese patients, so it was compelling to examine the associations between variations in this gene and metabolic complications of obesity. For instance, the reaction of myristoylation involves myristic acid, a saturated fatty acid,[10] which has been associated with the Western high-fat diet and related disease conditions.[26] Additionally, lipid-modified proteins may be integrated in the initiation of atherosclerosis.[27] A link between variants of this gene and obesity-related comorbidities is of interest. Moreover, an analysis of sequences coding for novel proteins potentially associated with MetS showed that they had numerous myristoylation sites,[28] suggesting that this type of modification can affect their activity. In this study, we reveal associations between genetic variations of the *NMT1* gene and components of MetS.

Considering that *NMT1* was found to be differentially expressed and methylated in VAT of MetS+ *vs.* MetS- obese men, associations of *NMT1* SNPs with obesity-related metabolic complications (BP, CRP, glucose and lipid levels) were tested.

SNP	cg16594296 Promoter	cg10755730 Promoter	cg21452443 Promoter	cg24354954 Promoter	cg07481784 Promoter	cg21554013 Promoter	cg02077631 Promoter	cg21132931 Promoter	cg22356484 Exon 1	cg06208294 Intron 1	cg00405568 Intron 1	cg09377882 Intron 1	cg09214551 Intron 1
rs2239921	0.80	0.39	0.47	0.57	0.71	0.06	0.21	0.38	0.55	0.62	0.63	0.53	0.59
rs2239923	0.12	0.03	0.31	0.45	0.81	0.44	0.14	0.51	0.78	0.60	0.77	0.98	0.34
rs2269746	0.76	0.28	0.51	0.99	0.34	0.83	0.20	0.74	0.72	0.44	0.56	0.93	0.17
rs8066395	0.93	0.41	0.90	0.48	0.50	0.63	0.67	0.82	0.04	0.68	0.79	0.76	0.59
rs2157840	0.15	0.57	0.37	0.19	0.12	0.68	0.18	0.78	0.89	0.97	0.45	0.46	0.43
rs1005136	0.15	0.57	0.37	0.19	0.12	0.68	0.18	0.78	0.89	0.97	0.45	0.46	0.43

SNP	cg03287877 Intron 1	cg08860622 Intron 1	cg00693004 Intron 1	cg04013970 Intron 1	cg02888886 Intron 3	ch_17_1184801R Intron 3	cg17942929 Intron 3	cg05349016 Intron 3	cg16080654 Exon 8	cg24136288 Exon 12 (3'-UTR)	cg22542420 Exon 12 (3'-UTR)	cg11583751 Exon 12 (3'-UTR)	cg05322982 Exon 12 (3'-UTR)
rs2239921	0.21	0.57	0.71	0.34	0.34	0.83	0.24	0.11	0.04	0.77	0.92	0.40	0.32
rs2239923	0.36	0.54	0.04	0.38	0.28	0.93	0.37	0.38	0.17	0.98	0.14	0.08	0.91
rs2269746	0.39	0.41	0.07	0.73	0.98	0.35	0.74	0.40	0.52	0.004	0.67	0.34	0.83
rs8066395	0.71	0.78	0.07	0.90	0.82	0.81	0.51	0.32	0.92	0.39	0.26	0.92	0.72
rs2157840	0.36	0.86	<0.0001	0.13	0.17	0.62	0.33	0.07	0.20	0.02	0.0004	0.41	0.71
rs1005136	0.36	0.86	<0.0001	0.13	0.17	0.62	0.33	0.07	0.20	0.02	0.0004	0.41	0.72

P values for associations were obtained from a subset of 14 obese subjects (7 MetS+ and 7 MetS-). CpG sites positions according to genome build 37.

Table 4: Association of phenotype-associated SNPs with gene methylation levels.

CpG site ID	Localization[a]	Region	Correlation coefficient[b]	*P* value	Number of individuals tested
cg10755730	chr17:43138257	Promoter	0.574	0.04	14
cg22356484	chr17:43138772	Exon 1	-0.079	0.81	13
cg00693004	chr17:43151433	Intron 1	0.277	0.36	14
cg16080654	chr17:43176852	Exon 8	-0.428	0.14	14
cg24136288	chr17:43183150	Exon 12 (3'UTR)	-0.008	0.98	14
cg22542420	chr17:43183176	Exon 12 (3'UTR)	-0.112	0.71	14

[a]CpG sites positions according to genome build 37. [b]Pearson's r correlation coefficient.

Table 5: Correlation of gene methylation with gene expression levels for SNPs-associated CpG sites in a subset of 14 severely obese subjects.

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Six gene polymorphisms of *NMT1* (rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136) were associated with obesity-related phenotypes. Our results suggest that *NMT1* is affecting inflammatory markers and blood pressure, as well as the plasma lipid profile. Thus, it contributes to the inter-individual variability observed in metabolic complications among obese patients. Although, no other study has considered *NMT1* as a candidate gene for the MetS. The involvement of *NMT1* in a genetic syndrome has, however, been demonstrated through the example of the N-myristoylated mutant form of the protein SHOC2 leading to the Noonan-like syndrome.[29] Whereas the wild-type protein is not myristoylated, a missense mutation in the SHOC2 gene results into its myristoylation, leading to the disorder. As proposed by Martin and coll,[25] this suggests that it is possible for other proteins that are not normally myristoylated to undergo this modification under certain circumstances and lead to damaging health conditions. Conversely, myristoylation can induce protection, such as in the case of cardiac ischemia-reperfusion injury where this protein modification protects against oxidation.[30]

To our knowledge, the present study is the first to present data suggesting a potential role for *NMT1* in lipoprotein and cholesterol metabolism, and it is particularly relevant as plasma lipid alterations are major elements of obesity and associated health risk factors.[31] Gene methylation and expression levels were thus examined to better understand the link between *NMT1* gene SNPs and MetS risk factors. Also, SNPs may influence gene methylation and expression levels.[32] Further, analysis were conducted with the six phenotype-associated SNPs and all of them were found to be significantly associated with at least one of the 26 CpG sites selected. This result suggests that *NMT1* SNPs affect gene methylation levels. Among the six significant CpG sites, only one (cg10755730) was significantly correlated with *NMT1* gene expression. The SNP rs2239923 was significantly associated with this methylation site ($p=0.03$), so it may be associated with *NMT1* gene expression. The link of *NMT1* phenotype-associated SNPs with gene methylation levels of CpG sites indicates a potential mechanism affecting gene expression and protein activity. Additional studies are needed to confirm the present findings and to better understand how *NMT1* SNPs, gene methylation and expression levels are linked to obesity and MetS.

Besides the absence of studies reporting associations between *NMT1* gene SNPs and plasma lipid levels, a possible link between *NMT1* and insulin has been put forward.[21,33,34] It is thus tempting to speculate that a possible mechanism relating *NMT1* to obesity and MetS might involve insulin. First, the known substrates of *NMT1* and insulin are indicated to be similar.[21] King, et al. has also suggested that *NMT1* is regulated by insulin, because the protein activity was observed to be inversely proportional to insulin levels in plasma.[21,34] As well, it has been reported that the insulin receptor can be myristoylated.[33] Afterwards, the observation that *NMT1* is overexpressed in MetS+ *vs.* MetS- obese subjects is in agreement with a possible insulin resistance associated with MetS. The demand of myristoylation by myristoylated proteins can modulate gene expression, like it is demonstrated in tumorigenesis.[35] Taken together, these observations would suggest that the differential expression of the *NMT1* gene between MetS+ and MetS- groups might be coming from an altered insulin pathway/function, and affect any of its constituents that need to be myristoylated. Indeed, insulin is considered to play a key role in the pathogenesis of the MetS,[36] even if it is not fully understood. Insulin resistant adipose tissue with limited expandability and lipid storage capacity eventually leads to systemic insulin resistance, involving of course other important sites of glucose uptake such as skeletal muscle, due to excessive postprandial non-esterified fatty acid spillover to non-adipose tissues and inflammatory mechanisms.[37] Hyperglycemia and dyslipidemia eventually emerge as major consequences of these alterations.[37] Similar mechanisms may also partly apply to hypertension which is related to obesity and insulin resistance.[38]

The present results support the potential role of *NMT1* in MetS. Nevertheless, this study is based on associations that need to take into account some potential limitations. First, gene methylation and expression in VAT were measured on a relatively small number of subjects. However, these analyses were only preliminary to find a potential mechanism relating gene variations to their associations with phenotypes. Additionally, for gene methylation and expression, VAT samples in their entirety were used for analyses, meaning that all cell types such as endothelial cells, fibroblasts and macrophages were included in addition to adipose cells. Between subjects differences in tissue composition could affect the results. Measurements on isolated cell fractions in future studies are needed. The results presented here were obtained by testing samples from severely obese individuals. This condition is known to modulate systemic inflammation[39] which, may also have altered the effects of *NMT1* SNPs.

CONCLUSION

Knowing that *NMT1* is overexpressed and hypermethylated in VAT of MetS+ compared to MetS- obese patients, the current study reveals the associations between SNPs within this gene and obesity-related metabolic complications. Specifically, SNPs of *NMT1* are associated with an altered lipid profile as well as with increased inflammatory marker levels and blood pressures. Additionally, the data related to gene methylation and expression levels suggest potential mechanisms linking *NMT1* gene variations to MetS risk factors.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests. AT receives research funding from Johnson & Johnson Medical Companies for studies unrelated to the present publication.

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AUTHORS' CONTRIBUTIONS

SBégin wrote the article; SBégin and FG completed the statistical analyses; MCV, AT, YD and LP established study design; SBiron, OL, LB and SM recruited patients, collected clinical data and samples; SBégin and MCV have principal liability for final content.
All authors read and approved the final manuscript.

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