ORIGINAL ARTICLE

Genetic regulation of differentially methylated genes in visceral adipose tissue of severely obese men discordant for the metabolic syndrome

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A genetic influence on methylation levels has been reported and methylation quantitative trait loci (meQTL) have been identified in various tissues. The contribution of genetic and epigenetic factors in the development of the metabolic syndrome (MetS) has also been noted. To pinpoint candidate genes for testing the association of SNPs with MetS and its components, we aimed to evaluate the contribution of genetic variations to differentially methylated CpG sites in severely obese men discordant for MetS. A genome-wide differential methylation analysis was conducted in visceral adipose tissue (VAT) of 31 severely obese men discordant for MetS (16 with and 15 without MetS) and identified ~17,800 variable CpG sites. The genome-wide association study conducted to identify the SNPs (meQTL) associated with methylation levels at variable CpG sites revealed 2292 significant associations ($P < 2.22 \times 10^{-11}$) involving 2182 unique meQTLs regulating the methylation levels of 174 variable CpG sites. Two meQTLs disrupting CpG sites located within the collagen-encoding *COL11A2* gene were tested for associations with MetS and its components in a cohort of 3021 obese individuals. Rare alleles of these meQTLs showed association with plasma fasting glucose levels. Further analysis conducted on these meQTL suggested a biological impact mediated through the disruption of transcription factor (TF)–binding sites based on the prediction of TF-binding affinities. The current study identified meQTL in the VAT of severely obese men and revealed associations of two *COL11A2* meQTL with fasting glucose levels. (Translational Research 2017;■:1–11)

Abbreviations: BMI = body mass index; CpG-SNPs = methylation-associated SNPs; DBP = diastolic blood pressure; GWAS = genome-wide association studies; HDL-C = HDL-cholesterol; HWE = Hardy-Weinberg equilibrium; LD = linkage disequilibrium; LDL-C = LDL-cholesterol; MAF = minor allele frequency; meQTLs = methylation quantitative trait loci; MetS = metabolic syndrome; NCEP-ATPIII = National Cholesterol Education Program Adult Treatment Panel III; SBP = systolic blood pressure; TF = transcription factor; TG = triglycerides; total-C = total-cholesterol; UTR = untranslated region; VAT = visceral adipose tissue; VEP = Variant Effect Predictor

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AT A GLANCE COMMENTARY

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Background

Genetic factors are involved in the establishment of DNA methylation levels at individual genomic loci and at the genome-wide level. Differences in methylation profiles of visceral adipose tissue (VAT) have been reported between men with vs without the metabolic syndrome (MetS). The current study evaluated the contribution of genetic variations to genome-wide methylation levels in VAT from severely obese individuals discordant for MetS.

Translational Significance

The current study demonstrates a contribution of genetic variation in differences in methylation levels observed between individuals discordant for MetS. Futhermore, it provides potential biological and mechanistic insights on the development of MetS through the identification of an association between *COL11A2* methylation-disrupting SNPs and fasting plasma glucose levels. Of foremost importance, it presents an analytical scheme integrating genotype, methylation, and expression data along with prediction of TF-binding affinities to potentially fill the gap of knowledge between associations reported and disease physiopathology.

INTRODUCTION

Epigenetic mechanisms are involved in the acquisition and maintenance of organized tissues[1] and represent a potential link through which genetics and environment may cause phenotypic variations.[2] Specifically, several pieces of evidence point to a contribution of genetic[3-5] and environmental factors in the establishment of DNA methylation levels at individual genomic loci or at the genome-wide level.[6,7] Alterations in DNA methylation are known to affect gene transcription, phenotypes, and methylation patterns associated with complex traits.[8,9] Changes in methylation levels at specific CpG sites have been demonstrated over time[6,10] and have been associated with multiple environmental factors.[7,11] Support for a genetic influence on methylation levels has emerged from estimates of methylation heritability in family as well as in twin studies,[12,13] and the identification of methylation quantitative traits loci (meQTL) in multiple samples and tissues.[3,4,14] Current evidence in the literature report a dependence of DNA methylation on local sequence content with associations observed close to the methylation site.[3,4,12,14] A striking impact of SNPs at methylation sites (hereafter termed CpG-SNPs) disrupting methylation potential and resulting in regions exhibiting allele-specific DNA methylation has been reported.[15]

Over the years, genome-wide association studies and candidate gene-based association studies identified several loci associated with complex traits including the metabolic syndrome (MetS),[16] a clustering of metabolic abnormalities defined by abdominal obesity, impaired glucose tolerance, dyslipidemia, and hypertension. Despite the fact that the heritability of MetS has been established,[17,18] genetic factors explain a small proportion of MetS variability. In line with the involvement of metabolically active tissues in the pathogenesis of complex diseases[19] and with the presence of tissue-specific methylation patterns,[20,21] our group previously established methylation profiles in visceral adipose tissue (VAT) of men with MetS (MetS+, N = 7) vs men without MetS (MetS−, N = 7) and revealed a potential association of specific metabolic pathways with the presence of MetS.[22]

The current study aimed to decipher the contribution of DNA sequence variations on methylation levels of severely obese men with vs without MetS. We extend our previous analysis of differentially methylated genes in VAT of individuals discordant for MetS by conducting an epigenome-wide association study for most variable sites. Focusing on genetic variations influencing the methylation status in VAT of men discordant for MetS, we present an approach integrating the genotype and methylation data to pinpoint the candidate genes for testing the associations of CpG-SNPs with MetS and its components. Through the analysis of CpG-SNPs identified in biologically relevant and metabolically active VAT, we provide a proof of concept for the identification of MetS-associated loci, thus integrating mechanistic insights to classic genetic association studies.

PATIENTS AND METHODS

Subjects. Blood samples and VAT were obtained as previously described[23] from a subset of 31 severely obese men (body mass index [BMI] >40 kg/m^2) undergoing biliopancreatic diversion with duodenal switch (BPD-DS)[24] at the Institut universitaire de cardiologie et de pneumologie de Québec - Université Laval (IUCPQ-UL) (Quebec City, Quebec, Canada). This subset of individuals, including 13 individuals previously analyzed for differences in methylation levels according to MetS group,[22] and not taking any medication to treat MetS features, was selected on the