Short Communication

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Bariatric surgery induces hypomethylation of genes related to type 2 Diabetes and insulin resistance

Abstract

Biliopancreatic diversion with duodenal switch (BPD-DS) is a surgical intervention known to induce substantial weight loss and significant long-lasting metabolic improvements including a decrease in insulin resistance (IR) and resolution of type 2diabetes(T2D). The specific mechanisms by which metabolic improvements occur after BPD-DS are still not fully elucidated and the impact of BPD-DS on gene methylation profiles has not been studied. To gain understanding of epigenetic factors that may predispose to metabolic improvements after weight loss surgery, we characterized the methylation signature of genes associated to T2D and IR after BPD-DS. Most of the genes involved in T2D and IR pathways exhibited significant differences in methylation levels after BPD-DS compared to a pre-surgery control group. The majority of these loci were significantly hypomethylated, suggesting an effect of bariatric surgery on the epigenetic signature of genes encoding proteins involved in glucose homeostasis.

Keywords: CPG sites methylation, bariatric surgery, epigenetics, type 2 diabetes, insulin resistance

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Abbreviations: BPD-DS, biliopancreatic diversion with duodenal switch; IR, insulin resistance; T2D, type 2 diabetes; VAT, visceral adipose tissue; CpG, cytosine-phosphate-guanite dinucleotide; BL, blood leukocytes; KEGG, kyoto encyclopedia of genes and genomes

Introduction

Biliopancreatic diversion with duodenal switch (BPD-DS) is a bariatric surgery inducing substantial weight loss and long-lasting metabolic improvements including, among others, improved insulin sensitivity and resolution of type 2 diabetes (T2D).[1]The specific mechanisms by which metabolic improvements take place after BPD-DS are still not fully elucidated. Among others, epigenetics has emerged as a novel mechanism potentially explaining part of the variability in the metabolic processes involved in glucose homeostasis.[2]

Knowledge of epigenetic factors determining the development of metabolic diseases remains largely incomplete. Previous studies have investigated the role of these factors in the severity of obesity-associated metabolic disturbances both at the whole-genome and candidate-gene levels. Previously, we reported that lower global methylation in visceral adipose tissue (VAT) may play a critical role in the development of obesity-associated metabolic alterations.[3] We also revealed that the epigenetic signature of VAT differed between obese men discordant for metabolic disturbances,[4] specifically within genes having a role in molecular pathways related to cell structure and cycle regulation, as well as in inflammation and immunity. We subsequently focused on two of the differentially methylated genes involved align those pathways, namely *ARPC3*[5] and *TOMM20*,[6] revealing a role of both genes in lipid management among individuals with severe obesity, potentially driven by an epigenetic-mediated mechanism. Concretely, this might occur through a differential methylation at cytosine-phosphate-guanine (CpG) sites located within gene promoter regions. We found that methylation of these loci potentially altered plasma triglyceride and cholesterol levels.

Studies carried out in subcutaneous adipose tissue (SAT) gave rise to similar findings to the aforementioned works in terms of affected metabolic pathways. A recent study performed in twins discordant for T2D and in an independent cohort of unrelated patients, found both epigenetic and transcriptional changes in key genes involved in lipid (*PPARG*) and also in carbohydrate metabolism (*IRS1*).[7] Likewise, fat cells from SAT of women with obesity also showed significant changes at epigenetic and gene expression levels mainly in adipogenesis, lipolysis and insulin signaling pathways.[8] Significant DNA methylation differences have also been found in other tissues than VAT or SAT. Davegårdh et al.[9] identified epigenetic and transcriptional changes in skeletal muscle between patients with versus without obesity. These changes were mainly observed in myogenic transcription factors, but also in other genes such as *IL-32*, which was further revealed as a novel target regulating insulin sensitivity. With a more precise focus on T2D, Dayeh et al.[10] showed that the epigenetic signature of pancreatic islets of T2D-patients was significantly different to that of islets from healthy donors, mainly at genes involved in insulin secretion and glucose homeostasis.[10,11] Altogether, these findings highlight the potential role of epigenetics in T2D pathogenesis and reveal that epigenetic changes taken place in obesity and T2D share common metabolic pathways and have a wide tissue distribution.

In this sense, we further broadened our research of VAT to more accessible blood leukocytes (BL). We hypothesized that some of the epigenetic determinants of BL associated with obesity-related complications may serve as surrogate markers of VAT.[12] Results of that study suggested that BL epigenetic signature may adequately reflect VAT methylation levels for genes associated with the development of obesity-related metabolic disturbances, specifically for genes encoding proteins involved in inflammation, and the metabolism of lipids and glucose.

Whether epigenetic profiling could identify predictors for the heterogeneous response to weight loss surgery also represents a new, barely studied paradigm. We have previously shown that epigenetic changes in BL could be involved in the metabolic profile improvement after weight loss surgery. Specifically, we revealed that offspring born after maternal BPD-DS surgery showed differentially methylated genes predominantly involved in glucoregulatory, immune, inflammatory and vascular diseases[13,14] Moreover, methylation levels, VAT mRNA abundance and markers of IR were significantly associated with metabolic improvements in offspring born after bariatric surgery.

More recently, to gain understanding of factors predisposing to metabolic improvements after weight loss surgery, we investigated the role of epigenetics in the resolution of T2D and related metabolic disturbances after weight loss surgery. Specifically, analysis of CpG sites methylation levels in women before and after BPD-DS revealed once again that diabetic and inflammatory/immune functions were among the most overrepresented in the list of genes with the largest methylation differences[15] Herein, our aim was to deepen our understanding of these epigenetic determinants in the metabolic improvements observed after BPD-DS by identifying the methylation signature of genes encoding proteins associated with T2D and IR.

Materials & methods

Briefly, whole-genome methylation levels at CpG sites were measured in blood DNA (Infinium Human Methylation 450 Bead Chips) of 20 women post-bariatric surgery (BPD-DS group) with a mean follow-up of 12years and in 20 pre-surgical women with severe obesity (control group). Participating women were matched for age, pre-surgery body mass index and metabolic parameters. A pathway-based differential methylation analysis was performed between the two groups for a set of genes encoding proteins involved in T2D and IR (T2D-IR genes), according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (IDs: 04930 and 04931). Differential methylation analysis was performed for 3,984 CpG sites, corresponding to 133 T2D-IR unique genes.[16]

Results

Anthropometric and metabolic parameters of study participants are summarized in Table 1. A total of 811 CpG sites belonging to 119 T2D-IRgenes (89.5% of genes) remained significantly different after Bonferroni correction ($P<1.43x10^{-7}$) (Figure 1a). Most differentially methylated CpG sites (60.8%) showed differences greater than 10%, and 97.2% of these sites were found to be significantly hypomethylated whereas 2.8% were over methylated in the BPD-DS group versus the control group (Figure 1b). The analysis of CpG sites within promoter regions revealed that five hypomethylated genes directly involved in insulin signaling (*INSR, PIK3CG, PTEN, IRS1* and *AKT2*) exhibited the largest differences in methylation levels between BPD-DS and the control group (Table 2).

Table 1 Characteristics of control and BPD-DS groups

Characteristics	Control	BPD-DS	P
Age (years)	29.2±3.3	41.0 ± 5.3	<0.0001
BMI (kg/m2)	45.8±5.7	27.6 ± 4.8	<0.0001
Blood pressure (mm Hg)			
SBP	130.0±12.4	112.2±9.5	<0.0001
DBP	81.3±8.6	68.2±8.9	<0.005
Lipid profile			
TC (mmol/l)	4.68±0.62	3.52±0.49	<0.0001
LDL-C (mmol/l)	2.79±0.58	1.68±0.50	<0.0001
HDL-C (mmol/l)	1.22±0.21	1.39±0.25	0.023
TG (mmol/l)	1.48±0.68	0.97±0.41	0.007
Glucose metabolism			
Fasting glucose (mmol/l)	5.2±0.9	4.7±0.3	0.038
Insulin (mU/mL)	26.6±23.9	3.1±1.7	<0.0001
HOMA-IR	5.9±5.0	0.7±0.4	<0.0001

Data is expressed as mean ± SD (n=20/group). BPD-DS, biliopancreatic diversion with duodenal switch; P, T-test P-value; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, Low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostatic model of insulin resistance

Table 2 List of 10 most differentially methylated CpG sites between the BPD-DS and control group

Rank	CpG ID	Position	UCSC gene	Localization	P	βDiff
1	cg14900579	19:7294137	*INSR*	TSS200	$1.11x10^{-16}$	-0.3
2	cg08779777	7:106505772	*PIK3CG*	TSS200	$2.22 x10^{-16}$	-0.188
3	cg06947206	10:89623157	*PTEN*	TSS200	$3.33 x10^{-16}$	-0.1
4	cg19358349	10:89621871	*PTEN*	TSS1500	$3.17 x10^{-13}$	-0.095
5	cg11620807	2:227664353	*IRS1*	TSS1500	$7.36 x10^{-38}$	-0.094
6	cg25333225	19:40791658	*AKT2*	TSS1500	$1.80 x10^{-13}$	-0.09
7	cg14157042	20:58515396	*PPP1R3D*	TSS200	$2.22 x10^{-16}$	-0.08
8	cg04471409	16:67040954	*RPS6KA2*	TSS1500	$1.46 x10^{-09}$	-0.079
9	cg20716209	17:40541270	*STAT3*	TSS1500	$1.20 x10^{-12}$	-0.071
10	cg01678714	5:179780409	*GFPT2*	TSS200	$1.10 x10^{-12}$	-0.062

CpG ID, cytosine-phosphate-guanine probe identification; Position, chromosomal and base pair position; UCSC Gene, university of california santa cruz genome browser gene mapping (GRCh37/hg19); localization, cpg localization relative to ucsc gene; P, P-value of differential methylation t-test; βDiff, average methylation β value difference

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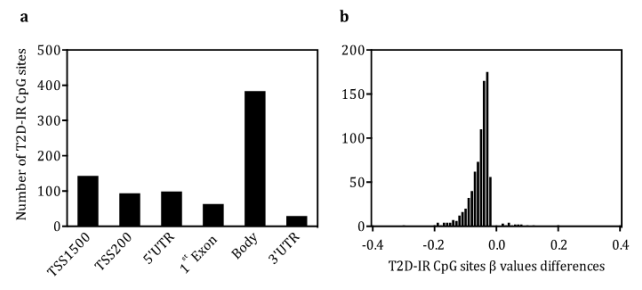

Figure 1 Differential methylation analysis a) Chromosomal distribution of T2D-IR CpG sites differentially methylated between BPD-DS and the control group (n=20/group). b) Histogram depicting β values differences in methylation levels between BPD-DS and control group (β>0 hypermethylation and<0 hypomethylation in BPD-DS group). TSS: transcription start site; UTR, untranslated region. CpG, cytosine-phosphate-guanine.

Discussion

Results from the present study show that most genes encoding proteins involved in T2D and IR pathways exhibited significant differences in methylation levels after BPD-DS when compared to a pre-surgery control group. Most of these loci were significantly hypomethylated, suggesting an effect of bariatric surgery on the epigenetic signature of genes involved in glucose homeostasis. Although there is no general consensus regarding the use of blood as surrogate of tissue-specific epigenetic changes,[17] a global conservation of DNA methylation profiles has been previously identified between blood and adipose tissue in obesity.[12,16] Accordingly, previous works have already reported differential hypomethylation induced by bariatric surgery in different tissues. Concretely, a recent study analyzing DNA methylation levels of isolated fat cells from SAT of women in a post-obese state also revealed a significant global hypomethylation,[18] with an over-representation of genes involved in adipogenesis. Similar results were found in both SAT and VAT,[19] as well as in skeletal muscle[20] in women after bariatric surgery. Interestingly, differential methylation at promoter regions was associated in such studies with gene transcription alterations, and even with improvements in glucose homeostasis clinical parameters, such as insulin sensitivity.[19,20] Thus, although further studies focused on the impact of post-bariatric hypo methylation in the transcriptional regulation of specific genes are still required, it is tempting to suggest that methylation changes found herein within T2D-IR-associated promoter loci may partly contribute to long-term effects of the BPD-DS on T2D resolution and improvements in IR.

Conclusion

Knowledge of epigenetic determinants predictive of obesity-related complications and metabolic improvements after bariatric surgery has considerable public health implications. Although further studies are still needed to establish a causal relationship between methylation changes in T2D-IR genes and metabolic outcomes after bariatric surgery, these epigenetic factors may be used as biomarkers to identify high-risk individuals that may be targeted for specific prevention and treatment programs, thereby reducing the burden of obesity and associated metabolic disturbances.

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Conflict of interest

Author declares that there is no conflict of interest.

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