Cell Metabolism
Short Article

DNA Methylation Analysis in Nonalcoholic Fatty Liver Disease Suggests Distinct Disease-Specific and Remodeling Signatures after Bariatric Surgery

Markus Ahrens,[1,12] Ole Ammerpohl,[2,12] Witigo von Schönfels,[1,12] Julia Kolarova,[2] Susanne Bens,[2] Timo Itzel,[7] Andreas Teufel,[7] Alexander Herrmann,[3] Mario Brosch,[3] Holger Hinrichsen,[8] Wiebke Erhart,[3] Jan Egberts,[1] Bence Sipos,[9] Stefan Schreiber,[3] Robert Häsler,[6] Felix Stickel,[10] Thomas Becker,[1] Michael Krawczak,[4] Christoph Röcken,[5] Reiner Siebert,[2] Clemens Schafmayer,[1,13] and Jochen Hampe[3,11,13,*]

[1]Department of General and Thoracic Surgery
[2]Institute of Human Genetics
[3]Department of Internal Medicine I
[4]Institute for Medical Statistics and Informatics
[5]Institute of Pathology
[6]Institute for Clinical Molecular Biology
Christian-Albrechts-University Kiel/University Hospital Schleswig-Holstein, 24015 Kiel, Germany
[7]Department of Internal Medicine I, University Hospital Regensburg, 93053 Regensburg, Germany
[8]Gastroenterology and Hepatology Center Kiel, 24105 Kiel, Germany
[9]Institute of Pathology, University Hospital Tübingen, 72074 Tübingen, Germany
[10]Department of Clinical Research - Hepatology, University of Berne, CH-3010 Berne, Switzerland
[11]Department of Medicine I, University Hospital Dresden, 01307 Dresden, Germany
[12]These authors contributed equally to this work
[13]Co-senior authors
*Correspondence: jochen.hampe@uniklinikum-dresden.de
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SUMMARY

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in industrialized countries. Liver samples from morbidly obese patients (n = 45) with all stages of NAFLD and controls (n = 18) were analyzed by array-based DNA methylation and mRNA expression profiling. NAFLD-specific expression and methylation differences were seen for nine genes coding for key enzymes in intermediate metabolism (including *PC*, *ACLY*, and *PLCG1*) and insulin/insulin-like signaling (including *IGF1*, *IGFBP2*, and *PRKCE*) and replicated by bisulfite pyrosequening (independent n = 39). Transcription factor binding sites at NAFLD-specific CpG sites were >1,000-fold enriched for ZNF274, PGC1A, and SREBP2. Intraindividual comparison of liver biopsies before and after bariatric surgery showed NAFLD-associated methylation changes to be partially reversible. Postbariatric and NAFLD-specific methylation signatures were clearly distinct both in gene ontology and transcription factor binding site analyses, with >400-fold enrichment of NRF1, HSF1, and ESRRA sites. Our findings provide an example of treatment-induced epigenetic organ remodeling in humans.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) describes a spectrum of liver disorders that occurs in the context of obesity and type 2 diabetes mellitus (Chalasani et al., 2012). While pure steatosis is a largely benign condition, it can be complicated by nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and liver failure. The pathogenesis of NAFLD is multifactorial and triggered by environmental factors such as hypercaloric nutrition and lack of physical activity in the context of genetic predisposition (Chalasani et al., 2010; Romeo et al., 2008). Bariatric surgery is the most radical therapy for the metabolic syndrome and NASH, leading typically to massive weight loss, improvement of liver histology (Dixon et al., 2004), and all-cause mortality (Lundell, 2012).

DNA methylation represents a level of epigenetic regulation that is closely linked to transcription factor (TF) binding and chromatin accessibility. While DNA methylation been studied intensively in cancer, including hepatocellular carcinoma (Ammerpohl et al., 2012), its pathogenetic role in benign disorders is only recently being recognized. DNA methylation signatures are not static but can be remodeled by TFs (Stadler et al., 2011) and by environmental stimuli (Barrès et al., 2012). The relevance of differential DNA methylation in NAFLD has been demonstrated for PPARGC1A, which showed a tight interaction to the insulin resistance phenotype (Sookoian et al., 2010) and by differential susceptibility of mice to hepatic steatosis (Pogribny et al., 2009) based on their epigenetic profiles.

Here we present a systematic analysis of DNA methylation in NAFLD and its dynamic remodeling after the massive weight loss induced by bariatric surgery.

RESULTS

Differences in DNA Methylation between Liver Phenotypes

Snap-frozen liver biopsies were obtained from 63 patients and classified histologically using the nonalcoholic fatty liver activity

Table 1. Sample Overview for the Discovery and Replication Samples

		Liver Phenotype Samples				Bariatric Samples		
		Normal Controls (C)	Healthy Obese (H)	Steatosis (S)	NASH (N)	Prebariatric (B1)	Postbariatric (B2)	Delta
Discovery	N methylation	18	18	12	15	n/a	23	n/a
	Age	51 [44–72]	44 [41–50]	46 [37–49]	47 [40–50]	47 [38–51]		n/a
	BMI	24 [21–26]	45 [42–49]	50 [47–55]	49 [44–56]	48 [45–54]	34 [30–40]	14 [12–16]
	Weight (kg)	67 [58–64]	135 [122–150]	147 [121–166]	146 [133–168]	146 [134–160]	106 [87–116]	−40 [−49– −34]
	Sex (% male)	50	0	42	27	17	17	n/a
	Diabetes (%)	11	17	25	20	26	17	−9
	Fat (area in %)	0 [0–1]	3 [0–4]	43 [20–70]	75 [70–85]	30 [12–70]	0 [3–25]	16 [5–40]
	Inflammation (0–3)	0 [0–0]	0 [0–0]	0 [0–0]	2 [1–2]	0 [0–1]	0 [0–0]	0 [1–0]
	Fibrosis (0–4)	0 [0–0]	0 [0–0]	0 [0–1]	1 [0–1]	0 [0–1]	0 [0–0]	0 [0–1]
	NAS (0–8)	0 [0–0]	0 [0–0]	2 [1–3]	5 [5–6]	2 [1–5]	0 [0–1.5]	2 [1–3]
	N mRNA (N overlap with methylation set)	12 (11)	16 (16)	9 (8)	17 (15)	n/a	16 (16)	n/a
Replication	N	10	9	10	10			
	Age	74 [66–77]	37 [34–43]	42 [31–59]	40 [35–47]			
	BMI	25 [23–26]	48 [46–51]	47 [40–57]	58 [57–59]			
	Weight (kg)	67 [62–72]	137 [128–144]	140 [117–162]	165 [159–186]			
	Sex (% male)	20	11	10	20			
	Diabetes (%)	20	0	40	20			
	Fat (area in %)	0 [0–0]	3 [2–5]	65 [31–78]	80 [70–82]			
	Inflammation (0–3)	0 [0–0]	0 [0–0]	0 [0–0]	1 [1–2]			
	Fibrosis (0–4)	0 [0–0]	0 [0–0]	0 [0–0]	1 [1–1]			
	NAS (0–8)	0 [0–0]	0 [0–1]	2 [1–3]	5 [5–6]			

The median and the interquartile range are provided for all numeric parameters. All prebariatric patients and no postbariatric patients are part of the liver phenotype samples. For the postbariatric samples, the change in the parameters is provided in the separate column. RNA for expression analysis was available for 70 liver samples, of which 66 were obtained from the same individuals who were used in the methylation experiment (in brackets). "Bariatric samples" refers strictly to patients for whom paired biopsies were available. The majority of the "liver phenotype samples" (H/S/N, 82%, 87%, and 90%) were obtained during bariatric surgery as well.

score (Kleiner et al., 2005) and clinically into normal controls (n = 18), healthy obese (n = 18), steatosis (n = 12), and NASH (n = 15). These biopsies are referred to as "liver phenotype samples." For 23 of these individuals (H/S/N: 7/10/6), follow-up liver biopsies 5–9 months after bariatric surgery were available. These postsurgery samples are referred to as "bariatric samples," bringing the total of analyzed liver biopsies to 86. The bariatric patients showed the expected improvement of liver histology (Dixon et al., 2004; Mathurin et al., 2006) (Table 1). All samples were assayed for CpG methylation at over 450,000 sites using an array-based approach. Array-based mRNA expression data were available for 70 liver samples, of which 66 were obtained from the same individuals as used in the methylation experiment (Table 1).

First, all sites deviating at least in one of the four phenotypic groups from the overall median methylation were identified using an omnibus (Kruskal-Wallis) test at a nominal significance of $p < 0.0001$. For the 273 CpG sites meeting this significance criterion, the medians of DNA methylation for each phenotype were sorted from lowest to highest. Under the null hypothesis, each of the 24 possible phenotype permutations would be equally abundant. However, particular ordered phenotype permutations were found to be strongly enriched ($p < 10^{-14}$, see Table S1A online), namely those compatible with a phenotypic progression from normal controls (C) to healthy obese (H), to steatosis (S), to NASH (N).

Second, we aimed to identify CpGs differentially methylated between phenotypic groups. In a global cluster and principal components analysis (PCA) at a false discovery threshold level of $q = 0.05$, 467 CpG loci were identified to be differentially methylated between the four phenotypic groups (online supplements can be found at http://gengastro.1med.uni-kiel.de/suppl/methyl_liver/). For ease of visualization, this analysis was repeated using $q = 0.004$, yielding 74 differentially methylated sites (Figure 1A). Both the heatmap and the PCA of these differentially methylated sites show normal liver samples and NASH as the extreme groups with healthy obese and steatosis samples located in the intermediate.

Expression Differences of Transcripts in the Liver Phenotype Comparison

Messenger RNA expression for the 294 genes annotated to the 467 CpGs differentially methylated between the four phenotypic groups was analyzed: 272 of these genes were present on the expression array. Analyses were restricted to CpG sites with at least a 5% difference of methylation between the phenotypic