Genome-wide DNA Methylome Alterations Are Associated With Histological Severity Of NAFLD-like Liver Injury Induced In Collaborative Cross Mice By An Obesogenic Diet

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in the United States and other countries and affects approximately one-quarter of the world's population. The complex heterogeneity of NAFLD is associated and influenced by multiple factors, including metabolic status, interindividual genetic predisposition, environmental factors, sex, ethnicity, nutrition, hormonal status, and microbiota. The role of disease-specific epigenetic alterations in the pathogenesis of NAFLD has been extensively studied, but epigenetic markers of individual susceptibility to the development of NAFLD remain unclear. In this study, Collaborative Cross mouse strains CC011/Unc (CC011) and CC042/GeniUnc (CC042) were fed a high-fat and high-sucrose (HF/HS) diet or a control diet for 12 weeks to investigate interindividual- and sex-specific DNA methylation alterations in the development of NAFLD. To investigate DNA methylation alterations induced by the obesogenic HF/HS diet, we performed genome-wide targeted DNA methylation next-generation sequencing analysis in the livers. The livers of male CC042 mice fed the HF/HS diet had a considerably greater number of differentially methylated regions as compared to that in male CC011 mice, and female CC011 and CC042 mice. Prevalent hepatic DNA hypermethylation changes occurred in male CC042 mice fed the HF/HS diet, while predominant DNA hypomethylation changes were specific for HF/HS diet-fed male CC011 mice and female CC011 and CC042 mice. The livers of CC011 and CC042 mice were characterized by substantial differences in cytosine DNA methylation and could be distinguished by their unique DNA methylation profile. In summary, the different extent and pattern of hepatic DNA methylation may play a key role in the susceptibility to the development of NAFLD.

Introduction

NAFLD is one of the most common chronic liver diseases in the United States and other countries, affecting approximately one-quarter of the world's population. NAFLD represents a spectrum of heterogeneous liver phenotypes ranging from nonalcoholic fatty liver (NAFL), or simple steatosis, to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis driven by metabolic dysfunction. Among the different mechanisms that drive NAFLD development and progression, epigenetic, especially DNA methylation changes, and gene expression alterations have been intensively studied in the past several years. However, a majority of the existing clinical reports provides only a snapshot of the altered DNA methylation and gene expression patterns at different disease stages in NAFLD patients rather than the dynamic changes that occur during the development and progression of NAFLD, making it difficult to clarify the role and functional significance of DNA methylation alterations in its pathogenesis. This limitation can be overcome in experimental studies by using animal models that mirror human NAFLD pathology.

In a previous study, using Collaborative Cross (CC) mice we demonstrated that feeding 25 male and 24 female CC mouse strains an obesogenic diet for 12 weeks resulted in the development of a NAFL-like phenotype in the livers of all mouse strains; however, the severity of hepatic steatosis varied across strains, with male CC042 mice being the most sensitive and male CC011 mice being the least sensitive. Based on those findings, the goal of the present study was to investigate alterations in DNA methylation in the livers of male and female CC011 and CC042 mouse strains that demonstrate differences in the magnitude of severity of NAFLD-like liver injury.

Materials and Methods

Animals and experimental design. In the present study, we used liver tissue samples of mice subjected to dietary model of NAFLD, in which male and female mice of twenty-five CC mouse strains were fed a HF/HS diet for 12 weeks.

Agilent SureSelect^{XT} Methyl-Seq targeted DNA methylation next-generation sequencing. The genomic libraries were prepared using the SureSelect^{XT} Methyl-Seq Library Preparation kits for targeted DNA methylation next-generation sequencing. Sequenced reads were processed using Trimmomatic and aligned to the mouse (Mus musculus) genome assembly GRCm38 (mm10) reference sequences that were bisulfite-treated in silico using Bismark Bisulfite Mapper with default parameters. Quantitative DNA methylation analysis and annotation was performed using SeqMonk tools.

Functional analysis of differentially methylated genes. Ingenuity Pathway Analysis (IPA) software was used for biological functional and pathway analysis of differentially methylated genes.

RNA-seq gene expression analysis. Total RNA libraries for RNA-sequencing were prepared using Illumina TruSeq Stranded Total RNA library preparation kits with Ribo-Zero Gold for rRNA depletion. The sequence reads were mapped to the mouse reference genome (NCBI38/mm10) using TopHat and Bowtie2 with default parameters. Transcript abundance estimates, normalizations, and differential gene expressions were generated using Cufflinks with default parameters.

Quantitative reverse transcription polymerase chain reaction. The expression of selected differentially methylated genes in the livers of CC mice was determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) using the TaqMan gene expression assays.

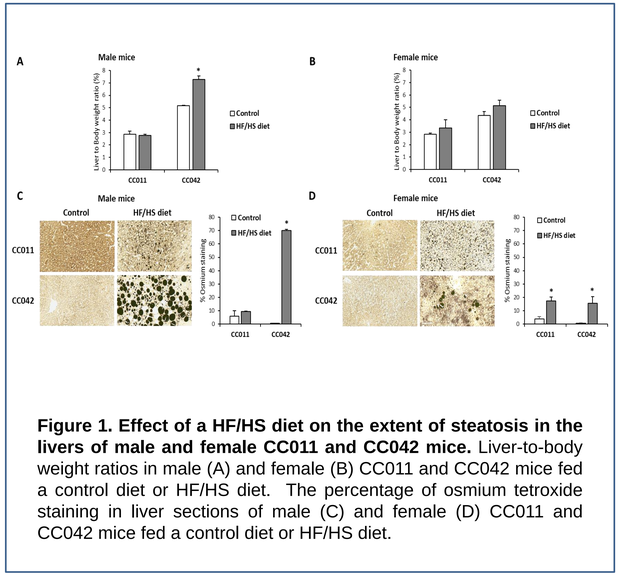

Figure 1. Effect of a HF/HS diet on the extent of steatosis in the livers of male and female CC011 and CC042 mice. Liver-to-body weight ratios in male (A) and female (B) CC011 and CC042 mice fed a control diet or HF/HS diet. The percentage of osmium tetroxide staining in liver sections of male (C) and female (D) CC011 and CC042 mice fed a control diet or HF/HS diet.

Results and Discussion

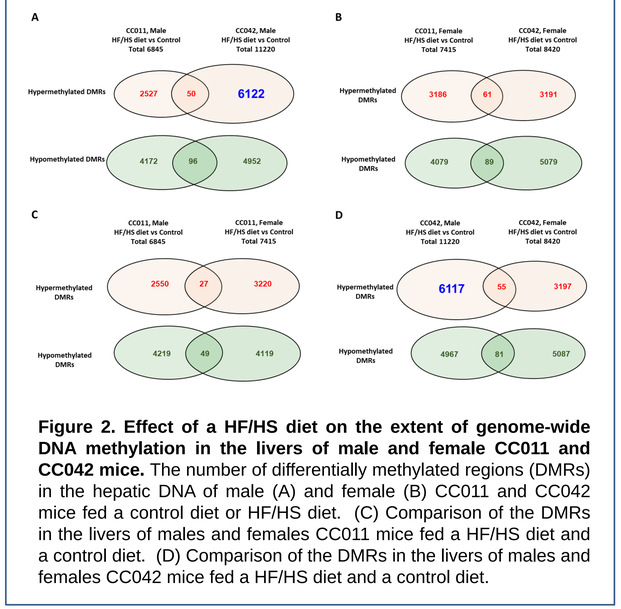

Figure 2. Effect of a HF/HS diet on the extent of genome-wide DNA methylation in the livers of male and female CC011 and CC042 mice. The number of differentially methylated regions (DMRs) in the hepatic DNA of male (A) and female (B) CC011 and CC042 mice fed a control diet or HF/HS diet. (C) Comparison of the DMRs in the livers of males and females CC011 mice fed a HF/HS diet and a control diet. (D) Comparison of the DMRs in the livers of males and females CC042 mice fed a HF/HS diet and a control diet.

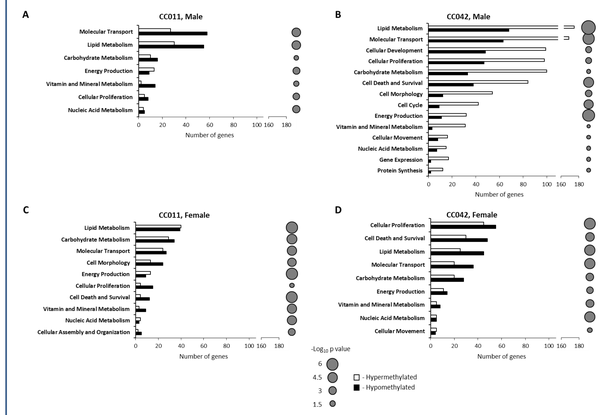

Figure 3. Pathway analysis of differentially methylated genes in the livers of male (A, B) and female (C, D) CC011 (A, C) and CC042 (B, D) mice.

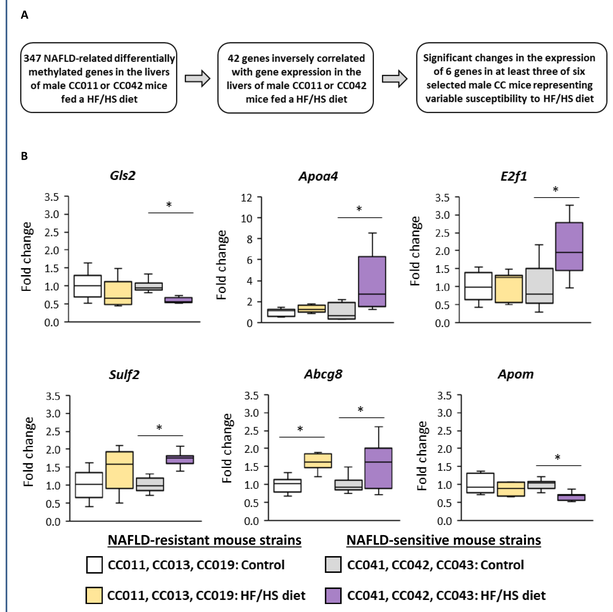

Figure 4. Identification of NAFLD-related DMR-containing genes in the livers of three sensitive to HF/HS diet-induced NAFLD-like liver injury male mouse strains, CC041, CC042, and CC043, and three resistant, CC011, CC013, CC019 mouse strains. (A) Algorithm of identification of NAFLD-related differentially expressed and DMR-containing genes. (B) Expression of NAFLD-related and DMR-containing genes in the livers of resistant and sensitive to NAFLD-like liver injury CC mouse strains.

Conclusions

- The results of our study demonstrate an association between the severity of NAFLD-like liver injury and magnitude of global DNA methylation changes.
- NAFL-like prone male CC042 mice demonstrated a global predominance of DNA hypermethylation, whereas a more pronounced DNA hypomethylation pattern developed in the mild-NAFL phenotypic male CC011 mice.
- The *Gls2*, *Apoa4*, *E2f1,* and *Apom* genes were differentially methylated and differentially expressed in the livers of NAFL sensitive mice.