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Research Paper

Systems Nutrigenomics Reveals Brain Gene Networks Linking Metabolic and Brain Disorders

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ABSTRACT

Nutrition plays a significant role in the increasing prevalence of metabolic and brain disorders. Here we employ systems nutrigenomics to scrutinize the genomic bases of nutrient–host interaction underlying disease predisposition or therapeutic potential. We conducted transcriptome and epigenome sequencing of hypothalamus (metabolic control) and hippocampus (cognitive processing) from a rodent model of fructose consumption, and identified significant reprogramming of DNA methylation, transcript abundance, alternative splicing, and gene networks governing cell metabolism, cell communication, inflammation, and neuronal signaling. These signals converged with genetic causal risks of metabolic, neurological, and psychiatric disorders revealed in humans. Gene network modeling uncovered the extracellular matrix genes *Bgn* and *Fmod* as main orchestrators of the effects of fructose, as validated using two knockout mouse models. We further demonstrate that an omega-3 fatty acid, DHA, reverses the genomic and network perturbations elicited by fructose, providing molecular support for nutritional interventions to counteract diet-induced metabolic and brain disorders. Our integrative approach complementing rodent and human studies supports the applicability of nutrigenomics principles to predict disease susceptibility and to guide personalized medicine.

1. Introduction

Metabolic disorders (MetDs) such as metabolic syndrome, obesity, and type 2 diabetes (T2D) have become a pressing health apprehension worldwide due to their increasing prevalence and high mortality rate, and even more recently to their ability to escalate the pathology of neurological and psychiatric disorders (Bomfim et al., 2012, Newcomer, 2007; Farooqui et al., 2012, Lowette et al., 2015). Among the potential culprits for the rising epidemic of metabolic and brain disorders are dietary components introduced through industrialization (Chassaing et al., 2015, Suez et al., 2014). In particular, fructose, which has been widely used as a "safe and healthy" sweetener in soft drinks and processed foods in the past decades, is emerging as a significant contributor to MetDs in humans (Lyssiotis and Cantley, 2013, Lustig et al., 2012). Fructose-induced MeDs has been shown to reduce hippocampal-dependent memory (Agrawal and Gomez-Pinilla, 2012) and to worsen the pathology of brain disorders in rodents (Agrawal et al., 2015). Conversely, the omega-3 fatty acid docosahexaenoic acid (DHA) has been shown to attenuate MetDs (Steffen et al., 2015, Virtanen et al., 2014, De caterina, 2011), and to counteract the deleterious effects of fructose on brain function and plasticity (Bremer et al., 2014, Agrawal and Gomez-Pinilla, 2012). Our understanding of the molecular mechanisms underlying the actions of fructose and DHA on MetDs and brain disorders has been limited by conventional approaches focusing on isolated molecular events. This limitation has delayed major advances

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in the utilization of nutrient-based strategies for the prevention and treatment of common complex disorders.

As fundamental aspects of gene regulation, disruptions in epigenomic reprogramming, transcript abundance, alternative splicing, and gene–gene interactions are increasingly recognized as core aspects of wide-ranging pathogenesis (Chen et al., 2008, Zhang et al., 2013, Rhinn et al., 2013, Narayanan et al., 2014, Makinen et al., 2014, Yang et al., 2009). Systems nutrigenomics is emerging as a powerful approach to reveal the hidden aspects of pathogenesis under dietary modulation (Zhao et al., 2015, De Graaf et al., 2009, Panagiotou and Nielsen, 2009). Here we apply systems nutrigenomics to unveil the multidimensional molecular interactions driven by fructose and DHA that regulate pathogenesis and recovery, and to provide proof-of-principle on the potential of systems nutrigenomics to guide personalized medicine. The comparative account of nutrigenomics signals between fructose and DHA is crucial to understand how select diets impact the molecular substrates governing the balance between normal brain function and disease, and holds potential for guiding effective preventative and therapeutic strategies to mitigate common human diseases.

2. Materials and Methods

We describe essential methods in the main text and detailed experimental procedures are available in the Supplemental Materials.

2.1. Overall Study Design

As depicted in the analysis flow in Fig. 1, we focus our study on two key regions in the rodent brain that are important for the regulation of metabolism (hypothalamus) and cognition (hippocampus), and therefore can play a major role in fructose-induced metabolic and brain dysregulation as well as DHA-mediated recovery. We analyzed the transcriptome and DNA methylome using next generation sequencing, followed by investigation of the regulatory relationship between the methylome and transcriptome. Network approaches were then applied to model gene–gene interactions and to predict essential perturbation or regulatory points. Knockout mouse models were subsequently used to validate the predicted regulatory genes with regard to their ability to modulate metabolic and behavior phenotypes. To infer translatability to human pathophysiology, we assessed the intersection of the molecular signals from our rodent models with human genome-wide association studies (GWAS) of metabolic and brain disorders.

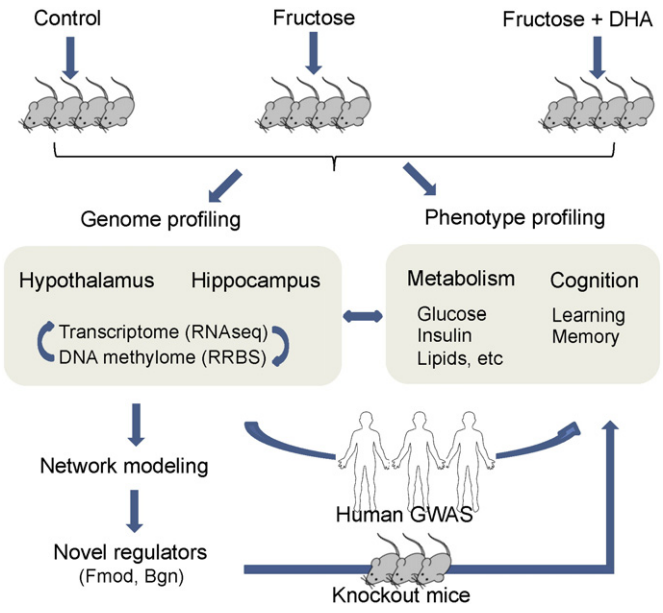

Fig. 1. Overall study design and analysis flow.

2.2. Rat model of fructose consumption and DHA supplementation

Male Sprague–Dawley rats (Charles River Laboratories, Inc., MA, USA) of 2 months old weighing 200-220 g were randomly assigned to 15% fructose treatment (n = 8, 15% *w/v* fructose in the drinking water), 15% fructose plus an omega-3 fatty acid diet rich in DHA (n = 8; 0.5% of flaxseed oil supplying ALA and 1.2% of DHA capsule oil, Nordic Naturals, Inc., CA, USA), or a control group (n = 8, without fructose in drinking water or DHA supplement) for six weeks. Sample size was chosen to yield >80% statistical power to detect 30% between-group difference with 10% within-group difference in a phenotype using two-sided Student's *t*-test. The rats were singly housed in polyacrylic cages with free access to water and their respective diets, and maintained under standard housing conditions (room temperature 22–24 °C) with 12 h light/dark cycle. Daily food and drink intake were monitored. The fructose intake level is approximately equivalent to long-term daily consumption of 130 g sugar in 1–2 l soda drinks in a 60 kg human. The total fat content in the control and DHA diets was 10 g per 100 g of diet. The rats were then examined for changes in MetD-related phenotypes (serum levels of insulin, glucose, and triglycerides, and insulin resistance index (fasting glucose [mg/dl] × fasting insulin [ng/ml] / 16.31)). Rats were trained in the Barnes maze test for 5 days to learn the task prior to diet treatment and then tested for memory retention in the Barnes Maze after 6 weeks of treatment as previously described (Agrawal and Gomez-Pinilla, 2012). Mice were sacrificed, and hypothalamus and hippocampus were dissected out, flash frozen, and stored at −70 °C for transcriptome and DNA methylome sequencing experiments.

2.3. RNA Sequencing (RNA-Seq) and Data Analyses

RNA-Seq libraries were prepared for 24 RNA samples (n = 4 per treatment group per brain region) and sequenced in paired-end mode by HiSeq 2000 (Illumina Inc., CA, USA) as detailed in Supplemental Materials. The short reads data were analyzed for different transcription between treatment and control groups using the Tuxedo tool package (Trapnell et al., 2012) and false discovery rate (FDR) was estimated using the q-value approach. One hypothalamus sample from the fructose group failed standard quality control and was removed from analysis. Genes and transcripts showing differential expression or alternative splicing at $p < 0.01$ in each brain region were defined as a gene "signature" for further integrative analyses. The reliability of transcriptome signals at $p < 0.01$ from RNA-Se1 was confirmed using quantitative real-time PCR (qPCR) for 12 selected genes (Supplemental Materials). Relative gene expression was represented by delta Ct = $Ct_{gene} - Ct_{Gapdh}$. The RNA-Seq data was deposited to Gene Expression Ominbus (GEO) under accession numbers GSE59918 (control and fructose groups) and GSE 64815 (DHA).

2.4. Reduced Representation Bisulfite Sequencing (RRBS) of DNA Methylome

RRBS libraries were constructed for 24 DNA samples (n = 4 per treatment group per brain region) and sequenced using an Illumina HiSeq 2500 System (Illumina Inc., CA, USA) as described in Supplemental Materials. Bisulfite-converted reads were processed using the bisulfite aligner BS Seeker2 (Guo et al., 2013). One hypothalamus sample from the DHA group failed standard quality control and was removed from analysis. Differential methylation between treatment and control groups was calculated using the R package methylKit (Akalin et al., 2012) and FDR was estimated using the q-value approach. Loci with methylation levels >25% between groups and FDR < 0.05 were defined as differentially methylated loci (DMLs). The potential *cis*- and