NAFLD in Hispanic Children: Evidence for Gene (PNPLA3) by Environment (dietary sugars/fructose) Interaction

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Hispanics Suffer from Dual Disparity to Obesity Starting in Early Life and Related to Early Exposure to Dietary Sugars

Shih et al; Pediatric Obesity 2013

The Main Dietary Driver of Obesity in Hispanic Children: High Sugar Sweetened Beverages

Davies et al; AJCN 2000

Link between high SSB and obesity established early in life
Among obese, odds of suspected NAFLD increased with age, BMI, Mexican American and male sex.

Trends in Adjusted Prevalence of Suspected NAFLD

Dietary Sugars and Liver Fat

47 adults
1Liter per day for 6 months
Monkeys exposed to ad lib fructose diet (24% of kcal) in the absence of weight gain

endotoxemia and microbial translocation

A

B

increase in liver fat

C

D

The Role of Fructose In Gut Permeability and Bacterial Translocation

Mice

- Fructose increased portal blood endotoxin levels and liver fat (antibiotic treatment protective)
- Suggests fructose and gut microbes play role in liver fat accumulation

Primates

- Fructose increased liver fat and damage
  - Secondary to increased microbial translocation from gut and circulating bacterial-derived lipopolysaccharides (LPS)

Human

- Fructose may induce intestinal permeability, LPS in portal blood, and liver fat (NAFLD)
- Fructose readily taken up by liver
  - Metabolism is unregulated
  - Contributes to formation of liver fat

Phyla Level: Diversity and Proteobacteria Associated with SSB/J Consumption

Proteobacteria:
- In adults, proteobacteria has been shown to be the most abundant bacteria phylum in the blood and predict cardiovascular disease
- Greatest abundance in the gut of those with NASH (6%), followed by obese (3%) and lean (<1%) children 13-14 years of age
Phyla Level: Diversity and Proteobacteria Associated with FRUCTOSE Consumption in Obese Hispanic Children (Preliminary data)

\[ r = 0.84; P<0.001 \]

OTU Level: Diversity and Sphingomonas Associated with SSB/J Consumption

\[ r = 0.38; P=0.007 \]

**Sphingomonas (Proteobacteria)**
- Efficient carbohydrate scavenger, sugars used for growth
- Associated with infections and immune responses
- Elevated in obese rats (accompanied by low Bifidobacterium [Actinobacteria])

Methods Development: Liver and Pancreas Fat at 3T

Iterative Decomposition using Echo-Asymmetry in the Least squares sense (IDEAL)

an optimal fat-water signal separation technique that utilizes knowledge of 1H spectra in lipid and water - an extension of NMR except it is multi-voxel and 3-dimensional across entire liver
Visceral, Liver and Pancreatic Fat Fraction by Ethnicity Pre-diabetes Status

Claudio Toledo-Coral PhD Student

Additional Genetic Risk Factors for NAFLD
Effects of PNPLA3 on Liver Fat and Metabolic Profile in Hispanic Children and Adolescents

Michael J. Goran, Bryan Walker, Kim-Anne Le, Benjamin Makhoul, Suzanne Vlacho, Joelio N. Davila, Donna Szynski, Max J. Wiersgowski, and Haveman Allens

Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption

AJCN, 2010

Clinical Characteristics

<table>
<thead>
<tr>
<th>Trait</th>
<th>All Participants (n=231)</th>
<th>Non-NAFLD (n=192)</th>
<th>NAFLD (n=39)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.3 ± 2.9</td>
<td>13.5 ± 2.1</td>
<td>13.3 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>99/137</td>
<td>87/104</td>
<td>12/25</td>
<td>0.005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.9 ± 28.2</td>
<td>72.1 ± 27.4</td>
<td>85.4 ± 27.6</td>
<td>3.4e10</td>
</tr>
<tr>
<td>BMI (kpl/m²)</td>
<td>20.5 ± 7.6</td>
<td>28.2 ± 7.1</td>
<td>33.4 ± 7.4</td>
<td>1.7e10</td>
</tr>
<tr>
<td>SAT (l)</td>
<td>12.1 ± 7.1</td>
<td>10.4 ± 6.6</td>
<td>14.2 ± 7.2</td>
<td>2.7e10</td>
</tr>
<tr>
<td>VAT (l)</td>
<td>1.8 ± 1.3</td>
<td>1.4 ± 0.9</td>
<td>2.2 ± 1.4</td>
<td>3.8e10</td>
</tr>
<tr>
<td>Total Fat (kg)</td>
<td>29.1 ± 12.1</td>
<td>26.1 ± 11.9</td>
<td>32.9 ± 11.5</td>
<td>5.9e10</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>8.8 ± 8.5</td>
<td>3.7 ± 1.1</td>
<td>15.2 ± 9.4</td>
<td>5.6e10</td>
</tr>
<tr>
<td>ALT (iu/l)</td>
<td>14.0 ± 10.2</td>
<td>10.0 ± 5.4</td>
<td>20.7 ± 12.3</td>
<td>2.2e10</td>
</tr>
<tr>
<td>AST (iu/l)</td>
<td>20.8 ± 8.2</td>
<td>17.8 ± 4.4</td>
<td>24.8 ± 10.3</td>
<td>4.4e10</td>
</tr>
<tr>
<td>TAG (mg/dl)</td>
<td>170.8 ± 122.7</td>
<td>96.8 ± 65.5</td>
<td>212.9 ± 55.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>140.0 ± 29.6</td>
<td>138.4 ± 28.6</td>
<td>143.3 ± 30.7</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>37.7 ± 9.4</td>
<td>36.0 ± 10.1</td>
<td>35.9 ± 8.3</td>
<td>0.924</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>85.2 ± 28.4</td>
<td>84.4 ± 28.8</td>
<td>88.2 ± 28.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Liver Fat Fraction by Ethnicity & Genotype

- -ve for gene
- -/+ for gene
- +ve for gene

Goran et al; Diabetes 2010
Data are shown as mean liver fat content (%) ± SD as a function of carrying 0, 1, or 2 copies of the effect allele for selected SNPs. Effects are shown as additive. Effect allele frequency in Hispanics. Effect allele frequency in Caucasians (HapMap-CEU). p-values are obtained from multiple linear regression using natural log-transformed values, adjusted for age, sex, and VAT.

Variants Associated with NAFLD in Hispanic Children and Adolescents

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Alleles</th>
<th>EA^f</th>
<th>Reported EA^f</th>
<th>Effect Allele Copy Number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>p^f</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNPLA3</td>
<td>rs196460</td>
<td>AG</td>
<td>0.53</td>
<td>0.039</td>
<td>0.843 ± 0.022</td>
<td>1.204 (n=35)</td>
<td>1.204 (n=80)</td>
<td>1.046 (n=35)</td>
<td>0.2267</td>
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<tr>
<td>APOC3</td>
<td>rs2854177</td>
<td>AG</td>
<td>0.35</td>
<td>0.021</td>
<td>0.464 ± 0.025</td>
<td>0.942 (n=100)</td>
<td>0.942 (n=80)</td>
<td>0.942 (n=100)</td>
<td>0.008</td>
</tr>
<tr>
<td>SCKR</td>
<td>rs419686</td>
<td>AG</td>
<td>0.32</td>
<td>0.021</td>
<td>0.844 ± 0.020</td>
<td>0.917 (n=100)</td>
<td>0.917 (n=80)</td>
<td>0.917 (n=100)</td>
<td>0.64</td>
</tr>
<tr>
<td>NCAN</td>
<td>rs72298109</td>
<td>TC</td>
<td>0.01</td>
<td>0.090</td>
<td>0.844 ± 0.020</td>
<td>0.917 (n=100)</td>
<td>0.917 (n=80)</td>
<td>0.917 (n=100)</td>
<td>0.003</td>
</tr>
<tr>
<td>LYPAT1</td>
<td>rs1213769</td>
<td>GT</td>
<td>0.92</td>
<td>0.719</td>
<td>0.844 ± 0.020</td>
<td>0.917 (n=100)</td>
<td>0.917 (n=80)</td>
<td>0.917 (n=100)</td>
<td>0.96</td>
</tr>
<tr>
<td>PPAR1R3B</td>
<td>rs4234324</td>
<td>AG</td>
<td>0.87</td>
<td>0.592</td>
<td>0.844 ± 0.020</td>
<td>0.917 (n=100)</td>
<td>0.917 (n=80)</td>
<td>0.917 (n=100)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Evidence of Interaction Between PNPLA3 and Dietary Sugar

This Concept is Supported by a Recent Study from the Hobbs lab using PNPLA3 knockin

PNpla3J48M Knockin Mice Accumulate PNPLA3 on Lipid Droplets and Develop Hepatic Steatosis

Eiko Sugaya, Sooam Biomedical, Holex Ltd, Yongbing Huang, Luen Lui, Yung Hsiung, Jonathan C. Gelet, and Benny H. Hobbs.

HERIDIOLOGY Vol. 41, No. 3, 2015
Plausible Biological Mechanism?

Other Dietary Factors Associated with Fatty Liver: Non-starchy Vegetable Intake

Obesity and NAFLD in Hispanics: The Perfect Storm

childhood obesity 7 times higher in low-income communities relative to affluent

Greater susceptibility to this environment in Hispanics starting in first 2y of life

From a dietary perspective, this effect is most related to high dietary sugars esp proliferation of HFCS in the diet

50% prevalence of PNPLA3 gene in Hispanics related to ~2-fold higher liver fat

Impact of PNPLA3 gene on fatty liver exacerbated by high sugar diet
Will Reducing Dietary Sugars in Hispanics be an Effective Intervention Strategy?

Good News and Bad News

The Bad News: 
Incredibly Difficult to Persuade Study Sections and NIDDK of Potential Impact of this Approach

R01 DK 091578-01A1; NIDDK - May 2011

PLA3 Genotype for Reducing Liver Fat in Hispanics with Pediatric Non-alcoholic Fatty Liver Score = 25; 8th percentile

"Unfortunately, your application was deemed to be of low programmatic priority"

Other Grants Not Funded
R21: Omega-3 Fatty Acid Supplementation for Treatment of Fatty Liver in Children
R01: Treatment of Insulin Resistance through Reduction of Liver Fat in Minorities
R01: Improving obesity and metabolic outcomes in Hispanics through maternal-infant intervention
R01: Improving obesity, liver fat and obesity outcomes in obese Hispanic teens through SSB reduction

The Graph:

Baseline 1 Year 2 Year

Body Weight (kg)

Treatment period Follow-up period

Hispanic Treatment Hispanic Control Non-Hispanic Treatment Non-Hispanic Control

zero effect in Non-Hispanics ~10kg reduced weight gain in Hispanics sustained over 2 years

Eibeling et al, NEJM 2012
Obesity, Diet, and the Gut Microbiota

Western Diet = High Fat/Sugar
- Obesity
- Fatty Liver (NAFLD)
- Changes in the gut microbiota

Cox AJ et al., Lancet Diabetes Endoc, 2014
Payne AN et al., Obes Rev, 2012