Genetics of NAFLD: what we know so far

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Heritability of NAFLD

- Heritability of NAFLD in minority cohorts (African Americans and Hispanics) was estimated to be 35%. Wagenknecht LE et al. Obesity 2009
- Fatty liver is a complex disease, whose heritability has been estimated to be around 40%. Schwimmer JB et al. Gastroenterology 2009
- Heritability of hepatic fibrosis and steatosis based on a prospective twin study has been estimated to be about 50%. Loomba R et al. Gastroenterology 2015

35%—40%—50%

Gene Variants and NAFLD

Gene variants associated with Intra-hepatic fat content by GWAS and hypothesis driven studies

GWAS studies
- PNPLA3
- LIPC
- LPRR4
- SLC38A8
- PARVB
- FDX1
- MTP
- ADRB
- FABP
- PPAR-alpha
- PPAR-gamma
- PPAR-beta
- AGTR1

Candidate genes studies
- APOC3
- LIPIN1
- FATP5
- MTHFR
- TNF-alpha
- TGF-beta

Findings replicated in pediatric populations

- Romeo S et al. Nature Genetics 2008
- Chalasani N et al. Gastroenterology 2010
- Speliotes EK et al. PLoS Genetics 2011
- Adams LA et al. Hepatology 2013
- Kitamoto T et al. Human Genetics 2013
- Kozlitina J et al. Nature Genetics 2014
**Gene Variants and NAFLD**

Gene variants associated with intra-hepatic fat content by GWAS and hypothesis-driven studies

**GWAS studies**
- GC
- LDLR
- LP
t
- SLC38A8
- SAMM50
- PPP1R3B
- PNPLA3
- PPP1R2B
- LPPR4
- PPP1R2A
- TCF7L2
- MTP
- ADRB1
- ADIPOQ
- PEMT
- PNPLA3

**Candidate genes studies**
- LCP1
- LPPR4
- SLC38A8
- FFAT5
- NCP5
- NFAT1
- TCF7L2
- ADRB1

Findings replicated in pediatric populations

**Role of PNPLA3 in NAFLD**

Genetic variation in PNPLA3 confers susceptibility to non-alcoholic fatty liver disease

The non-synonymous SNP rs738409 in the PNPLA3 is characterized by a C to G substitution encoding an isoleucine to methionine substitution at the amino acid position 148

**Role of PNPLA3 in NAFLD**

The Patatin-like phospholipase domain-containing protein 3 (PNPLA3) also known as adiponutrin (ADPN), is expressed in the liver and in the adipose tissue and has both triacylglycerol hydrolase and acyglycerol transacylase activity.
Role of PNPLA3 rs738409 in NAFLD

Impaired hydrolysis
Increased formation of TG

PNPLA3 rs738409  Hepatic fat content and Triglycerides

% Liver Fat Content

Triglycerides (mg/dl)

Caucasians  African Americans  Hispanics

p<0.0001  p<0.0001  p<0.01
p=0.38  p=0.69  p=0.72


I148M patatin-like phospholipase domain-containing 3 gene variant
and severity of pediatric nonalcoholic fatty liver disease

severity of liver steatosis  NASH  Fibrosis

Modified from Valenti L et al Hepatology 2010
The **PNPLA3 rs738409 SNP** modulate the degree of liver injury in other hepatic diseases

- Liver injury in NAFLD
- Alcoholic Fatty Liver Disease
- Liver damage in HBV and HCV
- Hepatocellular carcinoma in non-viral hepatitis
- NASH and Fibrosis in HIV-1-Monoinfected Adults
- Reduces Survival of patients with primary Sclerosing Cholangitis
- Liver damage in subjects with Inflammatory Bowel Disease
- Favors the onset of fibrosis in subjects with Hemochromatosis.

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**Gene Variants and NAFLD**

Gene variants associated with Intra-hepatic fat content by GWAS and hypothesis driven studies

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**GCKR gene and NAFLD**

- Speliotes BK et al. PloS Genetics 2011
GCKR gene and NAFLD

Chromosome 2p23.3

rs1260326
C1337T
(P446L)

GCKR gene and NAFLD

The binding affinity between GKRP for GK is reduced in subjects carrying the risk allele

Wild type

P446L GKRP

GCKR activity (% control)

Fructose 6-Phosphate (uM)

HEPATOCYTE

Large VLDL

Mitochondria

Nucleus

Lipid Droplets

> 5 mM/L

Glycogen

Glycolysis

Increased GK activity in the liver is predicted to enhance glycolytic flux, promoting hepatic glucose metabolism and elevating concentrations of malonyl-CoA, a substrate for de novo lipogenesis.

Fasting DNL% and Absolute Lipogenesis According to the GCKR 1260326 genotype

GCKR rs1260326 Phenotype
**GCKR rs1260326 Phenotype**

![GCKR rs1260326 Phenotype Diagram]

**Gene Variants and NAFLD**

Gene variants associated with Intra-hepatic fat content by GWAS and hypothesis driven studies

- **GWAS studies**
  - PNPLA3
  - GCKR
  - NCAN
  - PPP1R3B
  - LYPLAL1
  - TM6SF2
  - GC
  - LCP1
  - LPPR4
  - SLC38A8
  - SAMM50
  - PARVB
  - FDFT1

- **Candidate genes studies**
  - PNPLA3
  - GCKR
  - NCAN
  - PPP1R3B
  - LYPLAL1
  - TM6SF2
  - AGTR
  - MTP
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**TM6SF2 gene and NAFLD**

![TM6SF2 gene and NAFLD Diagram]

**TM6SF2 gene and NAFLD**

- **HEPATOCYTE**
  - Golgi body
  - TM6SF2
  - Large VLDL


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**TM6SF2 gene and NAFLD**

- **A**
  - Caucasians
  - P = 0.001

- **B**
  - African Americans
  - P = 0.02

- **C**
  - Hispanics
  - P = 0.0001

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**TM6SF2 gene and NAFLD**

- **A**
  - P = 0.95

- **B**
  - P = 0.04

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**TM6SF2 gene and NAFLD**

- **CC**
  - (n=155)
- **CT**
  - (n=28)

- **CC**
  - (n=116)
- **CT/TT**
  - (n=5)

- **CC**
  - (n=134)
- **CT**
  - (n=16)

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**Caucasians**

- **African Americans**

- **Hispanics**

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**ABC**

- **TM6SF2 gene and NAFLD**

- **CC**
  - (n=6)
- **CT**
  - (n=5)

- **Fibrosis stage ≤ 1**
  - Fibrosis stage > 1

- **P = 0.04**

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**Fibrosis stage (%)**

- **CC**
  - Fibrosis stage ≤ 1
  - (n=6)
- **CT**
  - Fibrosis stage > 1
  - (n=5)

- **P = 0.05**

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**NAFLD Activity Score**

- **P = 0.04**

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**Caucasians**

- **African Americans**

- **Hispanics**

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**Fibrosis stage (%)**

- **CC**
  - Fibrosis stage ≤ 1
  - (n=6)
- **CT**
  - Fibrosis stage > 1
  - (n=5)
**Gene Variants and NAFLD**

Gene variants associated with Intra-hepatic fat content by GWAS and hypothesis driven studies

<table>
<thead>
<tr>
<th>GWAS studies</th>
<th>Candidate genes studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC LPPL1</td>
<td>MTP ADIPOQ</td>
</tr>
<tr>
<td>LPP4 APOC3</td>
<td>ADIPOQ LPPL1</td>
</tr>
<tr>
<td>SLC35A1</td>
<td>FATP5</td>
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<td>PPAR-alpha</td>
</tr>
<tr>
<td>PARVB</td>
<td>PPAR-gamma</td>
</tr>
<tr>
<td>TM6SF2</td>
<td>HFE</td>
</tr>
</tbody>
</table>

Findings replicated in pediatric populations

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**TM6SF2 gene and NAFLD**

- Caucasians
- African Americans
- Hispanics

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Large VLDL (mg/dl)</th>
<th>LDL particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>40's-50's</td>
<td>60-80</td>
<td>20-40</td>
</tr>
<tr>
<td>60's-70's</td>
<td>80-100</td>
<td>40-60</td>
</tr>
</tbody>
</table>

p=0.03
p=0.04
p=0.015
p=0.004

**TM6SF2 gene and CVD Risk**

- CC
- CT/TT

Modified from Dongiovanni P et al. Hepatology 2015

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**Gene Variants and NAFLD**

Gene variants associated with Intra-hepatic fat content by GWAS and hypothesis driven studies
Two SNPs, C-482T and T-455C, in complete LD, in the insulin response element of the APOC3 gene.

As a result, carriers of the APOC3 risk alleles have increased hepatic uptake of lipids from chylomicron remnants, predisposing them to nonalcoholic fatty liver disease.
**APOC3 gene and NAFLD**

Genetic and Clinical Markers of Elevated Liver Fat Content in Overweight and Obese Hispanic Children

Ryan W. Walker, Frank Szapary, Diana Hartwig, Marc Wagenknecht, Donna Spruill-Milett, Tanya L. Wilkens, Michael S. Gorlov, and Sherman Meyers

![APOC3 rs2854117](image)

Modified from Walker RW et al. Obesity 2013

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**SNPs reproducibly associated with pediatric Fatty Liver**

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Function</th>
<th>Hepatic Fat</th>
<th>Circulating Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNPLA3</td>
<td>rs738409</td>
<td>remodeling of lipid droplets</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>GCKR</td>
<td>rs1260326</td>
<td>modulation of lipogenesis</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>TM6SF2</td>
<td>rs8542926</td>
<td>modulation lipoprotein secretion</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>APOC3*</td>
<td>rs2854117</td>
<td>modulation TG clearance</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

*Association found in Asian Indians and Hispanics

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![Lipid Droplets](image)

HEPATOCYTE

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How much variance of intra-hepatic fat content do these variants explain in the pediatric population?

About 40% of NAFLD heritability remains unexplained

Summary

- GWAS and candidate genes studies have allowed to discover gene variants associated with NAFLD, but only few of those have been replicated in pediatric populations.
- These studies have allowed to learn about genes, whose function was unknown.
- The majority of the SNPs associated to NAFLD is in genes involved in lipid metabolism.
- Altogether, the gene variants reproducibly associated with NAFLD in the pediatric population explain just a small fraction of NAFLD heritability.
- There is need for more genetic studies to discover new variants that may lead to the discovery of novel mechanisms underlying the pathogenesis of NAFLD.

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Learn and Live

African Americans
Hispanics
Caucasians

GWAS and candidate genes studies have allowed to discover gene variants associated with NAFLD, but only few of those have been replicated in pediatric populations. These studies have allowed to learn about genes, whose function was unknown. The majority of the SNPs associated to NAFLD is in genes involved in lipid metabolism. Altogether, the gene variants reproducibly associated with NAFLD in the pediatric population explain just a small fraction of NAFLD heritability. There is need for more genetic studies to discover new variants that may lead to the discovery of novel mechanisms underlying the pathogenesis of NAFLD.