**NASH BIOLOGY**

[Genetic contributions to NAFLD: leveraging shared genetics to uncover systems biology](https://www-nature-com.laneproxy.stanford.edu/articles/s41575-019-0212-0)

**General info:**

* NAFLD definition: fat accumulation in > 5% of hepatocytes without excess alcohol intake
  + Affects 1/4 of the global population
  + High heritability
  + No approved pharmacotherapy
  + Most common cause of liver disease in western countries
  + Projected to become the most common reason for liver transplantation
* Diagnosis:
  + Liver biopsy
  + Ultrasonography
  + MRI proton density fat fraction
  + Transient elastography
  + Blood tests - liver enzyme levels

[**Pathogenesis of Nonalcoholic Steatohepatitis: An Overview**](https://aasldpubs.onlinelibrary.wiley.com/doi/epdf/10.1002/hep4.1479)

* 2020
* NAFLD is the most common chronic liver disease in the US
* NAFL: excess fat deposition in liver that is unassociated with injury or inflammation
* NASH: hepatocyte ballooning, liver injury, inflammation, fibrosis
  + Leads to cirrhosis and HCC
* Histologic assessment:
  + Disease activity - scored on steatosis, ballooning, lobular inflammation
  + Fibrosis stage
    - Fibrosis associated with mortality
* Insulin resistance leads to reduced glucose uptake in adipocytes and muscles
* PNPLA3 encodes a lipid droplet (triglyceride storage) protein - regulation of lipolysis of lipid droplets
* Drug targets:
  + PPARa/b - increase fatty acid oxidation
  + FGF21 agonists
  + DNL inhibition
  + Increasing fatty acid desaturation
  + Improving IR - PPARy and glucagon-like peptide 1
  + Graphical user interface, text, application, email

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**Heritability:**

* Gene variants associated with susceptibility to NAFLD:
  + PNPLA3 - I148M
    - Most robust variant across entire spectrum of NAFLD
    - Implicated in lipid regulation
    - Also identified in alcoholic cirrhosis, hepatic steatosis in patients with viral hepatitis
    - Inhibition of PNPLA3 could limit hepatic steatosis but increase risk of CVD
  + TM6SF2 - E167K
    - Loss of function mutation
    - Regulates cholesterol synthesis and secretion of lipoproteins
    - Global inhibition of TM6SF2 to decrease serum lipid levels might prevent CVD but would cause hepatic steatosis and liver disease
  + GCKR
    - Variant that controls de novo lipogenesis by regulating influx of glucose into hepatocytes
    - Loss of function mutation coding for P446L variant
      * Variant regulates glucokinase in response to f-6-p and boosts lipogenic pathway - more substrates for fatty acid synthesis
  + MBOAT7
    - Encodes a protein in the Lands cycle
    - Identified in alcoholic cirrhosis, spectrum of NAFLD (including HCC), liver injury (inflammation and fibrosis) in patients with viral hepatitis
    - Highly expressed in inflammatory and immune cells
  + HSD17B13
    - Variant in this gene is associated with reduced serum liver enzyme levels and a reduced risk of NASH resulting from a production of an unstable protein with reduced function
    - Gene has been shown to have retinol dehydrogenase activity
  + Interferon  (IFN)-λ3/IFN-λ4 region:
    - Regulates innate immunity
    - Polymorphisms are associated with hepatic inflammation and fibrosis in patients with NAFLD and viral hepatitis

**Associated diseases:**

* T2DM
* CVD
* NAFLD loci are associated with increased risk of fatty liver and T2DM but decreased serum lipids and a reduced risk of CAD
* Family history of diabetes was significantly associated with NASH and fibrosis

**Therapeutic Targets:**

* FGF21 - a hormone secreted principally by the liver that regulates bile acid homeostasis
  + In phase 2 trials
  + Associated with reduced liver fat in patients with NASH

Notes:

* GWAS is unable to account for most of the heritability of NAFLD - 10-20%
* Shared gene effects between hepatic steatosis and related metabolic traits
* Diverging gene effects on various diseases
* Triglyceride levels depend on:
  + Hepatic production
  + Peripheral lipolysis
* **Altered retinol metabolism in the lipid droplets of HSCs/hepatocytes has been implicated in the pathogenesis of NAFLD and NAFLD-related fibrosis**
  + **Explains PNPLA3 and HSD17B13**

[Triggering and resolution of inflammation in NASH](https://www-nature-com.laneproxy.stanford.edu/articles/s41575-018-0009-6)

**NASH general info:**

* Characterized by steatosis, inflammation, and hepatocellular injury, fibrosis
* 1/3 of NASH patients might progress to advanced fibrosis or cirrhosis - HCC is a complication of cirrhosis
* Hepatic inflammatory response promotes sustained hepatic fibrogenesis, which leads to cirrhosis
* Probably preceded by chronic liver inflammation
* Steatosis = accumulation of triglycerides in the liver
  + Adaptive response to compensate for too many free fatty acids
* TG synthesis:
  + Increase FFA flow from AT/diet
  + Increased de novo lipogenesis in liver cells

**Causes of Inflammation:**

* Inflammation: response to tissue injury or infection that leads to secretion of cytokines, chemokines, and eicosanoids - coordinate cellular defense mechanisms and tissue repair

Overview:

* Increased visceral adipose tissue -> increased M1 macrophages -> adipose tissue insulin resistance, adipose tissue inflammation -> disturbed adipokine profile -> chemokines and cytokines are secreted from VAT macrophages -> liver inflammation, hepatic insulin resistance
* Increased FFAs due to adipose tissue lipolysis -> hepatic lipotoxic effects, cell death -> liver inflammation
* Dietary factors: induce lipotoxic effects, mitochondrial dysfunction, oxidative and endoplasmic reticulum stress and sterile cell death
  + Fructose, FFAs, free cholesterol, iron, low copper levels
* Resolution: shift to M2 macrophages, pro-resolving mediators (autacoids that act as stop signals of the inflammatory response and promote liver regeneration)

Diagram

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**Extrahepatic factors:**

Diet:

* Excess fructose:
  + stimulates de novo lipogenesis
  + Up-regulation of hepatic inflammatory genes
  + Reduction of hepatic mitochondrial b-oxidation and ATP levels
  + Fructose but not glucose induces NASH (zebrafish)
  + Disrupts mucosal membrane in gut -> bacterial overgrowth -> liver resident macrophages (kupffer cells) activated, increased TNF and lipopolysaccharide levels, MYD88 expression
* Metals:
  + Iron and copper homeostasis disturbance
  + Hepatic iron overload -> reactive oxygen species -> oxidative stress, immune activation, hepatocellular injury
  + Unclear - can trigger inflammatory mechanisms but do not lead to NASH most of the time
  + Low cellular copper -> elevated triglycerides and cholesterol
    - Copper deficiency induced NAFLD in rats
* Saturated fat, trans fat, cholesterol:
  + Saturated fatty acid intake -> de novo lipogenesis, expression of unfolded protein response related components, lipotoxicity
  + Trans fat induces NASH in mice
  + NASH -> cholesterol accumulation in kuppffer cells and hepatic stellate cells (which activate inflammation, fibrosis)
  + Polyunsaturated fatty acids -> lipid mediators that cause pro inflammatory AND anti inflammatory reactions (lipoxins - LXA4)
    - Lipid mediators from omega 3 fatty acids are pro-resolving, decrease lipogenesis
    - Interventional studies with omega threes haven't shown improvements in hepatocellular ballooning and fibrosis, but hepatic fat reduction
    - Oxidized metabolites from arachidonic acid and linoleic acid were associated with markers of steatosis, liver injury, insulin sensitivity, and secretion - more prevalent in plasma from patients with NASH than NAFL

Metabolic dysfunction

* Insulin resistance:
  + Occurs in most patients with NAFLD
  + Adipose tissue insulin resistance is critical in NASH development
* Adipose tissue dysfunction
  + Visceral adipose tissue releases macrophage produced chemokines and cytokines
    - CCL2 and TNF, IL-1B and IL-6
    - Increased mRNA levels of macrophage associated and inflammation associated genes in adipose tissue preceded their expression in the liver
  + Leads to inflammation, impaired insulin signalling
  + Depletion of kuppfer cells cannot reverse hepatic and systemic IR once obesity and inflammation in adipose tissue is established
  + CCL 2 promotes macrophage recruitment
  + AT IR is higher in patients with NASH
  + ADIPOKINES:
    - Adiponectin, leptin, TNF, and IL-6 drive NASH
    - Increased levels of leptin and decreased levels of adiponectin -> NAFLD disease severity
    - TNF increases insulin resistance and has pro inflammatory effects
    - IL-6 promotes IR
    - Leptin:
      * Circulating leptin is elevated in NAFLD
      * Leptin initially inhibits hepatic de novo lipogenesis but has an inflammatory function later on

Gut:

* Link between NASH and intestinal dysbiosis - gut barrier dysfunction
  + Endotoxins can induce inflammatory response through activation of liver inflammatory cells
  + MD2 and TLR4 deficiency gets rid of inflammation in liver in mice
  + Lack of TLR5 exacerbates liver disease
  + CX3CR1
    - Chemokine receptor 1
    - Controls gut barrier permeability
    - Deletion exacerbates steatohepatitis
  + JAMA
    - Tight junction protein
    - Deficiency -> increased gut permeability and bacterial translocation to the liver
    - NASH induction
* NAFLD patients have gut inflammation, decreased CD4+ and CD8+ T lymphocytes in intestinal mucosa
  + Gut cytokine levels and tight junction disruption
* Bile acids:
  + Regulate lipid and carbohydrate metabolism through FXR receptor and TGR5
  + Dysregulation can lead to alterations in glucose and lipid homeostasis and promote inflammation and fibrosis
    - Altered bile acid metabolism can affect microbiota composition and dysbiosis can affect bile ace
  + Increased bile acid exposure might be involved in liver injury and NASH pathogenesis
    - Defective hepatic bile acid transport

**Intrahepatic factors in NASH:**

Hepatocellular stress:

* Oxidative stress
  + Correlate with neutrophil numbers and degree of liver damage in human liver NASH samples
  + Reactive oxygen species -> disrputed lysosomal membranes -> release of proteases -> apoptosis and necrosis
  + Kuppfer cells are major source of ROS
  + HSCs produce ROS
  + Cytochrome P450 - oxidizes fatty acids, produces ROS
* ER stress
  + Mitochondria produce ROS
* Lipotoxicity
  + Triglyceride accumulation is a protective mechanism to counteract lipotoxicity
    - Inhibition of triglyceride synthesis in mice can reduce hepatic steatosis but exacerbate liver damage and fibrosis
    - Triglyceride accumulation is insufficient to cause IR and inflammation in the liver
  + Saturated fatty acids are directly toxic to cells
    - Activate JNK and mitochondrial death pathways, increase ER stress
  + Monounsaturated fatty acids might protect against toxicity
  + Excess free cholesterol can induce hepatoxicity - sensitizes cells to cell death signals such as TNF
    - Accumulates in kuppfer cells and hscs
* Apoptosis
  + TMBIM1 suppresses nash
    - Localizes in endosomes and lysosomes that regulate protein degradation
  + Apoptotic bodies can stimulate activation of HSCs, inducing fibrogenesis
    - TRAIL = death receptors
    - ASK1 - activates JNK and p38 MAPK pathways in response to stressors (ROS, ER stress, LPS, and CA2+ overload)
      * Triggers cell death, insulin resistance, inflammatory response, hepatic steatosis
    - CASP8, CFLAR, TNFAIP3 - suppressors of ASK1 activation that ameliorate NASH
    - Caspase 2 - upregulated in NASH - role in lipid induced hepatocyte apoptosis
* Necroptosis
  + RIP1, RIP3 regulate necroptosis
    - Increased RIP3 expression in NASH
* Pyroptosis
  + Pyroptosis = novel form of cell death that is dependent of caspase 1
    - Activation of inflammasomes and formation of pores in the cell membrane
  + NLRP3 activations promotes transition from steatosis to NASH in mice
    - NLRP3 inhibitor reduces liver inflammation and fibrosis in ANSH
    - NLRP3 are inflammasome particles released during pyroptosis
      * Spreads inflammation and inflammasome signalling to adjacent cells
* Autophagy
  + Downregulated in mice with NAFLD
    - May cause failure to remove damaged mitochondria
* Nuclear receptors
  + PPARa, PPARd, PPARy, LXRa, LXRb, FXR, PXR, CAR
    - Associated with energy and nutrient control
  + Down regulated NF-kB levels could be good for nash
    - Omega 3s activate PPARa and down regulate NF-kB but don’t prevent steatohepatitis development in mice
  + PPARg - attenuates inflammation
  + LXR - reduces hepatic steatosis
* Hepatokines:
  + Steatotic hepatocytes release hepatokines - induce pro inflammatory signalling and IR
* Adatptive immune system:
  + TLR2, TLR4, TLR9 overexpressed in NASH liver samples
    - TLR signalling induces activation of transcription factors that produce inflammatory cytokines, chemokines, type 1 interferons
  + Neutrophil peptides activae HSCs in mice and promote fibrosis
    - Neutrophil apoptosis failure contributes to inflammation in NASH

**Epigenetics/genetics:**

* Snps:
  + PNPLA3, TM6SF2, GCKR
  + MBOAT7-TMC4
  + APOB
  + LPIN1
  + UCP2
  + COL13A1
  + EFCAB4B
  + FDT1
  + MTTP
  + GCLC
  + IFNL4
* Differentially methylated genes - differentiate NASH from steatosis
  + FGFR2
  + MAT1A
  + CASP1

**Resolution of inflammation:**

* KCs = M1 and M2 macrophages
  + Switch in phenotype is critical for resolution of inflammation
* Specialized pro-resolving mediators
  + Promote resolutionof inflammation:
    - Clear neutrophils from inflamed tissue, stimulate recruitmentof nonphlogistic mononuclear cells, switching macrphophage phenotype
  + Derived from EPA and DHA
  + LXA4 - lipoxin analoge - helps adipose tissue inflammation
* Diagram

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Graphical user interface

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**PAPERS ON METHODS**

[**Integrating node embeddings and biological annotations for genes to predict disease-gene associations**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6311944/pdf/12918_2018_Article_662.pdf)

* Binary classification for disease gene prediction using embeddings and annotations as features
* Used node2vec embeddings concatenated with biological annotations from uniprot ("keywords") to predict gene-disease associations from OMIM
* Used feature selection to identify annotations relevant to each disease
  + Different feature selection by disease to predict
* Performed imbalance correction to correct for smaller # of genes associated with each disease
* SVM, random forest, kNN, GLM
* Similar to what we have done!!

[**Uncovering disease-disease relationships through the incomplete interactome**](https://science.sciencemag.org/content/347/6224/1257601)

* + Looked at overlap in disease-related gene subnetworks and found association between diseases that mirror comorbidity rates even without and obvious pathobiological relationship between the two diseases
  + Found that topological relationships in network related to biological relationships

[**Predicting Parkinson's Disease Genes Based on Node2vec and Autoencoder**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6454041/)

* Used SVM on node2vec embeddings learned from a PPI network to predict new Parkinson’s disease related genes
* Reduced dimension of the embedding vector through use of an autoencoder
* Randomly selected genes not associated with Parkinson’s disease to use as negative set
* New predicted genes were verified through literature search

[**Pathway and network embedding methods for prioritizing psychiatric drugs**](https://www.biorxiv.org/content/10.1101/728055v1.full.pdf)

* Yash and Margaret’s project
* Used gene expression data from a couple difference psychiatric conditions to predict diagnosis
  + PCA and UMAP to predict disease/no disease
* Used PROPS method to calculate pathway importance scores
  + Decision tree, SVM, random forest to predict which disease
  + Added to gene targets for each drug by extracting differentially expressed genes from CMap (expression data before and after treatment)
  + Combined disease gene signatures and drug-gene target lists to recommend drugs for a disease in ranked order
* Used node2vec on String and GNBR
  + Augmented drug recommendation list with mean pairwise cosine similarity of genes in disease module to genes in drug modules

[**Semantic Disease Gene Embeddings (SmuDGE): phenotype-based disease gene prioritization without phenotypes**](https://academic.oup.com/bioinformatics/article/34/17/i901/5093225)

* Created vector embeddings from STRING PPI
* Used cosine similarity of gene/disease embeddings - ranks gene-disease based on pairwise similarity of gene-disease (each disease has 1 embedding)
* Trained artificial neural network to predict gene-disease associations from embedding vectors
* Created a ranking classifier based on the model’s prediction scores, computed AUC

[**SemanticGO: a tool for gene functional similarity analysis in *Arabidopsis thaliana* and rice**](https://www.sciencedirect.com/science/article/pii/S0168945220301321?casa_token=ri5TrUHU4nMAAAAA:7XL8-g8IhTuxVPJWqyLAcgqLmfjgYhUk-MWG6bix-mCSjdBzXxuF00pRLRPXIluWgPV7ug)

* Used latent semantic analysis to create gene vectors from GO ontology labels on genes
* **Sums vectors of genes in a pathway to create a pathway representing vector**
* Computed the cosine similarity of vectors to determine association

[**Inferring novel genes related to oral cancer with a network embedding method and one-class learning algorithms**](https://www.nature.com/articles/s41434-019-0099-y)

* Embedded STRING PPI network using node2vec
* Used feature selection and built several different one class algorithm based inferring models

[Node2vec: SNAP biology tutorials](http://snap.stanford.edu/deepnetbio-ismb/)

* Useful info on node2vec and embeddings