# Nonalcoholic fatty liver disease; Pathogenesis

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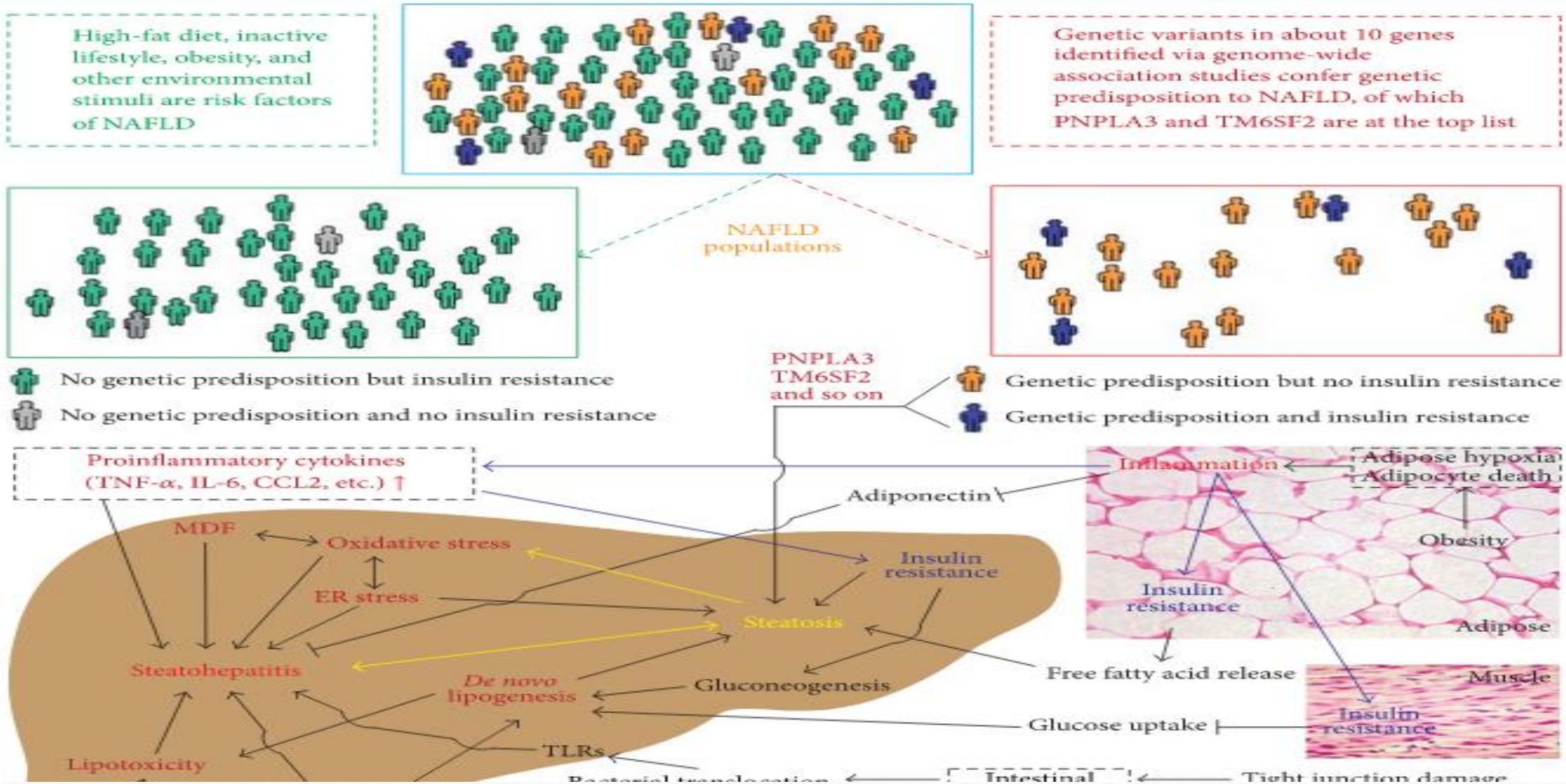
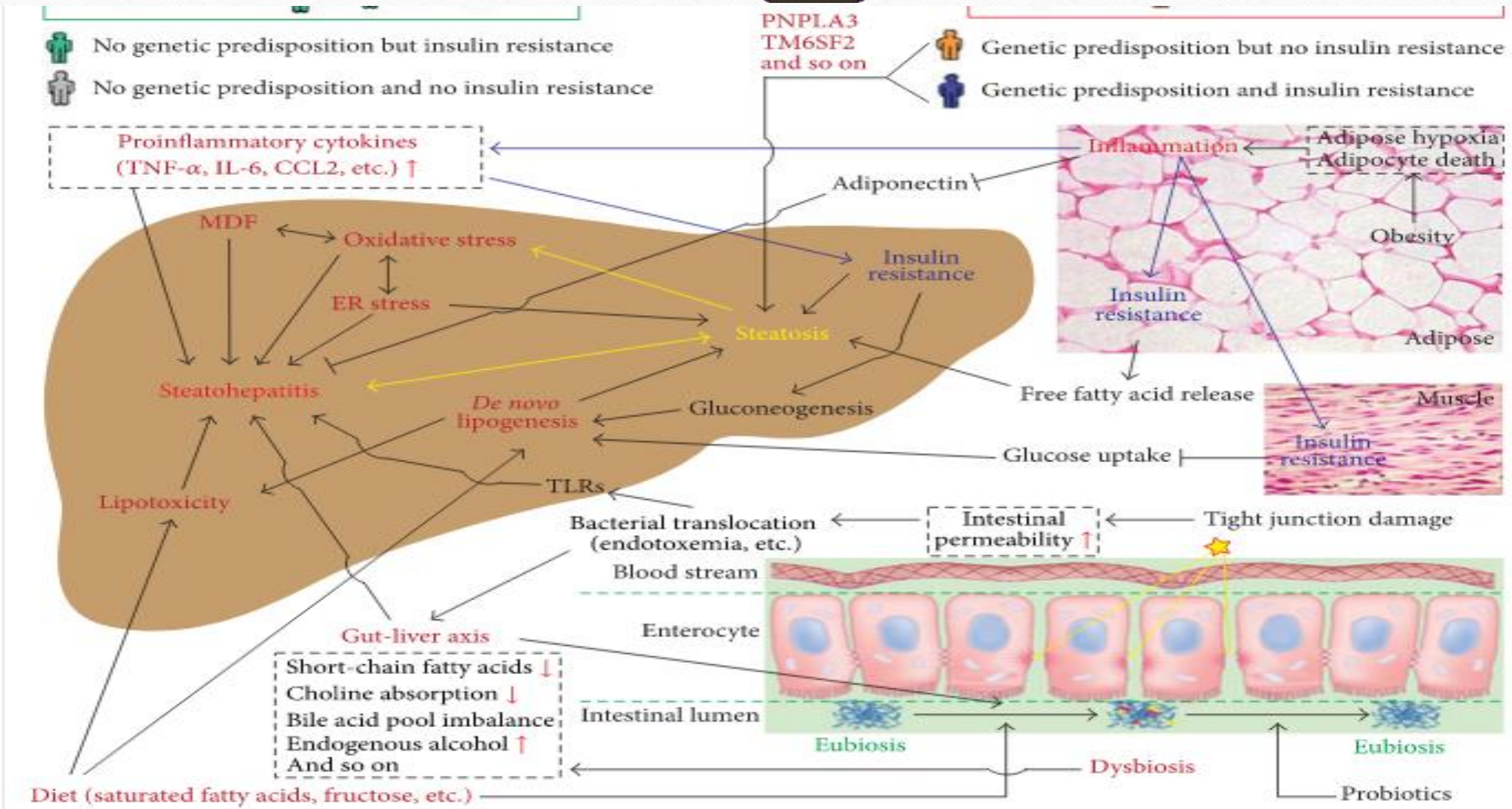
# Nonalcoholic fatty liver disease (NAFLD)

- The most common chronic liver disease in the world, with an estimated rising prevalence, in parallel with the epidemics of obesity and type 2 diabetes.
- It is present in 30% of the general adult population and found predominantly in obese people with high-fat diets and inactive lifestyles.
- In reality, NAFLD comprises a spectrum of hepatic abnormalities that are observable in liver histological slides, from a simple intrahepatic accumulation of fat (steatosis or nonalcoholic fatty liver, NAFL) to various degrees of necrotic inflammation (nonalcoholic steatohepatitis, NASH)

# Nonalcoholic fatty liver disease (NAFLD)

- Simple steatosis (i.e., NAFL) rarely progresses to advanced disease whereas, in approximately 20% of patients with NASH, it progresses to fibrosis and cirrhosis and potentially to hepatocellular carcinoma over a 15-year time period
- The majority of patients with NAFLD are obese or even morbidly obese and have accompanying insulin resistance that plays a central role in the metabolic syndrome
- NAFLD is also deemed to be hepatic manifestation of metabolic syndrome which is a cluster of complex conditions including central obesity, hypertension, hyperglycaemia, hypertriglyceridemia, and low HDL (high density lipoprotein) that are predictive risk factors of cardiovascular disease, stroke, and diabetes.


High-fat diet, inactive lifestyle, obesity, and other environmental stimuli are risk factors of NAFLD
Genetic variants in about 10 genes identified via genome-wide association studies confer genetic predisposition to NAFLD, of which PNPLA3 and TM6SF2 are at the top list
NAFLD populations
No genetic predisposition but insulin resistance
No genetic predisposition and no insulin resistance
PNPLA3
TM6SF2
and so on
Genetic predisposition but no insulin resistance
Genetic predisposition and insulin resistance
Proinflammatory cytokines (TNF-α, IL-6, CCL2, etc.) ↑
Inflammation
Adipose hypoxia
Adipocyte death
Adiponectin
MDF
Oxidative stress
ER stress
Insulin resistance
Steatosis
Obesity
Insulin resistance
Adipose
Steatohepatitis
Free fatty acid release
De novo lipogenesis
Gluconeogenesis
Muscle
Glucose uptake
Insulin resistance
TLRs
Lipotoxicity
Intestinal
Tight junction damage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Steatohepatitis
Free fatty acid release
De novo lipogenesis
Gluconeogenesis
Muscle
Insulin resistance
Glucose uptake
TLRs
Lipotoxicity
Bacterial translocation
(endotoxemia, etc.)
Intestinal permeability ↑
Tight junction damage
Blood stream
Enterocyte
Gut-liver axis
Short-chain fatty acids ↓
Choline absorption ↓
Bile acid pool imbalance
Endogenous alcohol ↑
And so on
Intestinal lumen
Eubiosis
Eubiosis
Dysbiosis
Diet (saturated fatty acids, fructose, etc.)
Probiotics

# Common Pathogenic Mechanisms of NAFLD

- Several mechanisms may lead to steatosis, including;
- (1) increased fat supply such as high-fat diet and excess adipose lipolysis;
- (2) decreased fat export in the form of VLDL-triglyceride;
- (3) decreased free fatty $\beta$-oxidation;
- (4) increased *de novo* lipogenesis (DNL)
- Molecular mechanisms responsible for the accumulation of fat in the liver are not fully understood; however, certain cytokines derived from inflammation sites, particularly from extrahepatic adipose tissues, can trigger this process.
- At a certain point, the simple steatosis transforms to steatohepatitis in about 20–30% of NAFLD patients.

# Adipose Tissue Inflammation

- Adipocytes under inflammation secrete cytokines and chemokines, particularly TNF-*a*, IL-6, and CC-chemokine ligand-2 (CCL2) .
- TNF-*a* was the first proinflammatory cytokine detected in adipose tissue and is involved in the regulation of insulin resistance.
- IL-6 is derived from many cells throughout the body including adipocytes& correlate remarkably well with the presence of insulin resistance,
- CCL2 recruits macrophages to the adipose tissue, resulting in even more local cytokine production and perpetuating the inflammatory cycle;
- Together, these abnormalities accentuate fat loss from adipocytes and promote ectopic fat accumulation.

# *De Novo* Lipogenesis (DNL)

- Presumably lipogenesis in liver could be increased due to the steatotic nature of NAFLD.
- Basically since carbohydrates are substrates for DNL, the amount of carbohydrate in the diet will positively influence the amount of DNL in the liver.
- Simple sugars are converted to fatty acids more readily than complex carbohydrates ,and fructose is a more potent inducer of DNL than glucose .
- It is worth noting that dietary fat, particularly saturated fat, stimulates DNL

# Insulin Resistance

- Is caused by a variety of factors, including soluble mediators derived from immune cells and/or adipose tissue, such as TNF-*a* and IL-6 .
- Insulin resistant subjects with NAFLD show reduced insulin sensitivity, not only at the level of the muscle, but also at the level of the liver and adipose tissue , which can lead to a far more complex metabolic disturbance of lipid and glucose.
- It is worth noting that insulin resistance is characterized not only by increased circulating insulin levels but also by increased hepatic gluconeogenesis, impaired glucose uptake by muscle, and increased release of free fatty acids and inflammatory cytokines from peripheral adipose tissues, which are the key factors promoting accumulation of liver fat and progression of hepatic steatosis.

# Lipotoxicity

- This is particularly true of the long-chain saturated fatty acids (SFAs) such as palmitate (C16:0) and stearate (C18:0), which are abundant in animal fat and dairy products and produced in the liver from dietary sugar.
- In addition, the toll-like receptor 4 (TLR4) is a pattern recognition receptor that activates a proinflammatory signaling pathway in response to excessive SFAs.

# Mitochondrial Dysfunction

- Mitochondria are the most important energy suppliers of the cell and play a pivotal role in fatty acid metabolism.
- Fatty acid oxidation is able to be upregulated to compensate for some degree of increased deposition of fat; however, multiple studies have shown that liver ATP levels are decreased in NAFLD.
- Although the mechanisms responsible for the mitochondrial dysfunction remain poorly understood in NAFLD

# Oxidative Stress

- In the context of increased supply of fatty acids to hepatocytes, oxidative stress can occur and be attributable to raised levels of reactive oxygen/nitrogen species (ROS/RNS) and lipid peroxidation that are generated during free fatty acid metabolism in microsomes, peroxisomes, and mitochondria.
- These reactive lipid derivatives have the potential to amplify intracellular damage by mediating the diffusion of ROS/RNS into the extracellular space, thus causing tissue damage.

# Endoplasmic Reticulum (ER) Stress

- The ER is a vast dynamic and tubular network responsible for the synthesis, folding/repair, and trafficking of a wide range of proteins.
- Such an imbalance between the load of needed protein-folding and the response-related capability of the ER is termed ER stress, which can lead to the accumulation of unfolded and/or misfolded proteins within the ER lumen.
- Coupled with inflammation, oxidative stress, insulin resistance, and apoptosis signaling, hepatic ER stress seems to play an important role in regulating the composition and size of lipid droplets as well as lipid synthesis, including cholesterol metabolism.

# Microbiota Associated Mechanisms of NAFLD

- Derangement of the gut flora, in particular small intestinal bacterial overgrowth (SIBO), occurs in a large percentage (20–75%) of patients with chronic liver disease.
- This approach allows the characterization of both composition and diversity of the intestinal microbiota.
- The gut microbiota may contribute to the pathogenesis of NAFLD through (1) increased production and absorption of gut short-chain fatty acids; (2) altered dietary choline metabolism by the microbiota; (3) altered bile acid pools by the microbiota; (4) increased delivery of microbiota-derived ethanol to liver; (5) gut permeability alterations and release of endotoxin; and (6) interaction between specific diet and microbiota.

# Short-Chain Fatty Acids (SCFAs) Relevant Mechanisms

- In the intestine, SCFAs are produced in the distal small intestine and colon where non-digestible carbohydrates like resistant starch, dietary fiber, and other low-digestible polysaccharides are fermented by saccharolytic bacteria which include the phyla Bacteroidetes, Firmicutes, and Actinobacteria. Acetate and propionate are the main products of Bacteroidetes phylum and butyrate is mainly produced by Firmicutes phylum. As an energy precursor, SCFAs are implicated in the pathogenesis of NAFLD because of their possible contribution to obesity.

# Dietary Choline Mechanism

- Dietary choline is required for VLDL synthesis and hepatic lipid export; and dietary choline-deficiency has been linked with a variety of conditions including hepatic steatosis.
- One study suggests a role of choline in fat export out of the hepatocytes.
- A metagenomic analysis of the microbial communities living in the intestinal tracts of 15 women with a choline-depleted diet revealed that increased Gammaproteobacteria abundance and decreased Erysipelotrichi abundance were protective against developing steatosis

# Bile Acid Pool Related Mechanisms

- Within hepatocytes, bile acids are synthesized from cholesterol through enzymatic pathways and then conjugated with either glycine or taurine before secretion into bile and released into the small intestine. In the small intestine, conjugated bile acids not only assist in lipid absorption and transport but have also been increasingly recognized to function as nuclear receptor binders and to have a putative role in altering the microbiome .
- Bacteria can also chemically modify bile acids and thereby alter the composition of the bile acid pool .
- Besides the classic role as detergents to facilitate fat absorption, bile acids have also been recognized as important cell signaling molecules regulating lipid metabolism, carbohydrate metabolism, and inflammatory response .??

# Endogenous Alcohol Theory

- Alcohol in the breath of obese animals is higher than that of lean animals , but they could not find any difference in the breath alcohol concentration between NASH patients and lean controls in a human study .
- NASH patients exhibited significantly elevated blood ethanol levels, while similar blood ethanol concentrations were observed between healthy subjects and obese non-NASH patients?? .
- Further, in this metagenomics study, the composition of NASH microbiomes was found to be distinct from those of healthy and obese microbiomes, and *Escherichia* stood out as the only abundant genus that differed between NASH and obese patients. Because *Escherichia* are ethanol producers, this finding is in agreement with their previous report that alcohol-metabolizing enzymes are upregulated in NASH livers .
- Thus, the alcohol theory currently faces conflicting results from different investigators. ??

# Intestinal Permeability and Endotoxemia

- The gut microbiota plays a part in maintaining the integrity of the intestinal barrier , and changes in the composition of microbiota can lead to increased intestinal permeability and subsequent overflow of harmful bacterial by-products to the liver that in turn triggers hepatic inflammation and metabolic disorders.

# Saturated Fatty Acids

- It has been well known that animal meats are rich in saturated fatty acids (SFAs) which are highly correlated to an increased risk of obesity, diabetes, and cardiovascular diseases. Many studies have indicated that SFA are more toxic than their unsaturated counterparts.
- It is worth noting that SFAs are protective in alcohol induced fatty liver disease .
- However, in liver and hepatocytes not exposed to alcohol, SFAs appear to promote apoptosis and liver injury .
- Such a typical obesity microbiota profile stimulated by SFAs favors the development of obesity and hepatic steatosis

# Fructose

- Fructose has been utilized as artificial sweetener in many commercial soft drinks that are consumed largely by adolescents and in a variety of social circumstances. A number of studies have found that excess fructose consumption is involved in the pathogenesis of NAFLD and that upregulated *de novo* lipogenesis and inhibited fatty acid $\beta$-oxidation are distinct metabolic processes for the development of hepatic steatosis in individuals with NAFLD.
- Increased fructose consumption is associated with a higher fibrosis stage in patients with NAFLD, independent of age, sex, BMI, and total calorie intake .
- Using a fructose-induced NAFLD mouse model found that fructose significantly decreased *Bifidobacterium* and *Lactobacillus* and tended to increase endotoxemia. Several probiotic bacterial strains of *Lactobacillus* protect mice from the development of high-fructose-induced NAFLD.

# Genetic Background of NAFLD

- Genomic variations that have a causative effect on the development of human diseases can be divided into two groups: ones in rare diseases and ones in common diseases.
- Among all reported genes, only two of them (*PNPLA3* and *TM6SF2*) have been identified as potential genetic modifiers in more than one large scale study.
- According to genotypes in those key genes and sensitivity to insulin, NAFLD patients can be categorized into different subpopulations (Figure 1).

# Interplay between Diet, Microbiota, and Host Genetics

- A high-fat diet and inactive lifestyles are typical risk factors for NAFLD, the interplay between diet, gut microbiota, and genetic background can play a crucial role in the development and progression of NAFLD.
- NAFLD is a multi-etiology disease trait, meaning that it is not caused by a single gene mutation genetically and is not associated with only a single factor environmentally; but it is the outcome of genetic variant-environmental factor interplay determining disease phenotype and progression.

# Conclusions

- The genetic variants in *PNPLA3* and *TM6SF2* are only responsible for ~50% of NAFLD patients , and majority of *PNPLA3*-associated NAFLD patients are not obese and have no insulin resistance and its related diabetes and cardiovascular diseases.
- NAFLD is polygenic, where the heritable component to susceptibility variously accounts for up to 30–50% of relative risk .
- Individual environmental factors, particularly the specific diets, interact with gut microbiota up front before a final beneficial or damaging signal is sent.
- Whether environmental factors, including lifestyle, are the cause of NAFLD will be steered by the interaction with the host genetics as well as the constitutional profile of gut microbiota.
- Given the multifaceted pathophysiology of NAFLD, probably, a combination of approaches in an individualized basis may be a more appropriate management.

- Thanks for your consideration,
- Any question will be appreciated