The Riddle of Nonalcoholic Fatty Liver Disease: Progression From Nonalcoholic Fatty Liver to Nonalcoholic Steatohepatitis

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Nonalcoholic fatty liver (NAFL) is an emerging global epidemic which progresses to nonalcoholic steatohepatitis (NASH) and cirrhosis in a subset of subjects. Various reviews have focused on the etiology, epidemiology, pathogenesis and treatment of NAFLD. This review highlights specifically the triggers implicated in disease progression from NAFL to NASH. The integrating role of genes, dietary factors, innate immunity, cytokines and gut microbiome have been discussed. (J Clin Exp Hepatol 2015;5:147–158)

Keywords: adiponectin, cytokines, gut microbiota, lipotoxicity, PNPLA3
Received: 14.11.2014; *Accepted:* 9.2.2015; *Available online:* 17.2.2015
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Abbreviation: AGE: Advanced glycation end products; ALT: Alanine aminotransferase; AMPK: AMP-activated protein Kinase; APPL1 and 2: Adaptor protein 1 and 2; ATP: Adenosine tri-phosphatase; BMI: Basal Metabolic Index; CD: Cluster of differentiation; COL13A1: Collagen, type XIII, alpha 1; DAMP: Damage assocauted molecular pattern molecules; EFCAB4B: EF-hand calcium binding domain 4B; FA: Fatty acid; FDFT1: Farnesyl-diphosphate farnesyltransferase 1; FFA: Free fatty acid; GCKR: Glucokinase regulatory protein; GLUT 5: Glucose transporter type 5; GWAS: Genome wide association studies; HDL: High density lipoprotein; Hh: Hedgehog; HMGB1: High-mobility group protein B1; HOMA-IR: Homoestatic model assessment-insulin resistance; HSC: Hepatic Stellate Cells; IL6: Interleukin 6; IR: Insulin Resistance; KC: Kupffer Cells; LPS: Lipopolysacharrides; LYPLAL1: Lypophospholipase like 1; MCP: Monocyte chemotactic protein; NAD: Nicotinamide adenine dinucleotide; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NCAN: Neurocan gene; NF-KB: Nuclear Factor Kappa B; NK: Natural Killer; NKL: Natural Killer T cells; NLR: NOD like receptor; NNMT: Nicotinamide N-methyltransferase gene; OXLAM: Oxidized linolenic acid metabolite; PAMP: Pathogen-associated Molecular pattern; PARVB: Beta Parvin Gene; PDGF: Platelet-derived growth factor; PNPLA3: Patatin-like phospholipase domain-containing protein 3; PPAR-α: Peroxisome proliferator activated receptor alpha; PPP1R3B: Protein phosphatase 1 R3B; PUFA: Poly unsaturated fatty acid; PZP: Pregnancy-zone protein; ROS: Reactive oxygen species; SAMM: Sorting and assembly machinery component; SCAP: SREBP cleavage-activating protein; SFA: Saturated fatty acid; SNP: Single nucleotide polymorphism; SOCS3: Suppressor of cytokine signaling 3; SOD2: Superoxide dismutase 2 gene; SREBP-1C: Sterol regulatory Element—Binding Protein 1-C gene; TLR: Toll like receptor; TNF α: Tumor necrosis factor Alpha; UCP3: Uncoupling protein 3 gene

http://dx.doi.org/10.1016/j.jceh.2015.02.002

Non alcoholic fatty liver disease (NAFLD) is an emerging medical problem worldwide which affects a significant proportion of the western population and there is gradual spread of this epidemic to south-east Asian countries. NAFLD encompasses two entities: Non-alcoholic fatty liver (NAFL) and Non-alcoholic steatohepatitis (NASH). NAFL is defined as the evidence of hepatic steatosis without inflammation either by imaging or by histology in individuals without significant alcohol consumption in whom secondary causes of steatosis are absent.[1] NASH on the other hand, is characterized by the presence of both steatosis and inflammation with evidence of hepatocyte injury in the form of ballooning with or without fibrosis.[1]

The prevalence of NAFLD has been gradually increasing and one third adult Americans have NAFLD.[2] The prevalence in obese population may be as high 57.55–74%.[3,4] The global spread of this epidemic is evidenced by the presence NAFL in 36.8% of Mediterranean, 21.5% of Iranians and 27% of urban Chinese adults.[5–7] However, the prevalence varies between countries and continents. The prevalence in Europe is 20–30% while that in Japan varies between 9 and 30%[8,9] and in China between 5 and 24%.[10] In India, the prevalence of NAFLD in urban population is 16%–32% while that in rural areas is approximately 9%.[11–13] Among the Asian countries the lowest prevalence is observed in Singapore at 5%.[10] Globally NAFLD has been related to obesity and sedentary lifestyle. Interestingly both NAFL and NASH have been observed in non-obese subjects in Asians, which is referred to as the Asian paradox.[11,14,15] Mere presence of fat in the hepatocytes is not considered as a disease. As most of the subjects with NAFL do not progress to NASH, differentiation between these two conditions is paramount. Though liver histology is the

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gold standard in diagnosis of NAFL, the commonest method used is transabdominal ultrasonography which has a sensitivity of 100% and specificity of 90% when fat on liver biopsy exceeds 20%.[16] Other non-invasive modalities used to diagnose NAFL includes transient elastography (Fibroscan) and Acoustic Resonance Magnetic Imaging (ARFI) which measures liver stiffness and corresponds to presence of fibrosis. Magnetic resonance spectroscopy is a quantitative method of measuring liver fat but is limited in clinical use due to lack of widespread availability and cost.[17] However, none of these available noninvasive tests can distinguish simple steatosis from NASH. Although raised aspartate aminotransferase and alanine aminotransferase levels are considered by some as a marker of NASH, yet these enzymes may be normal in many subjects with biopsy proven NASH.[18] A recent study using non-invasive tools have found 81% probability of differentiating NAFL from NASH using Bayesian approach combining clinical, laboratory, and imaging data.[19] Based on the modality used to diagnose NAFL, the detection of disease varies. Ultrasound based study from India has shown the prevalence of NAFLD to be 16.6%[12] while in a study based on liver biopsy the presence of NASH was 53%.[20] In another study from costal eastern India, one fourth of patients had evidence of NASH on liver biopsy on presentation.[21] In another Asian study involving 52 patients, biopsy proven NASH at presentation was found in 32.6% patients while 23% patients with baseline NAFL progressed to NASH.[22] In contrast, a recent study from the West with mean 6.6 years follow up, baseline NASH was found in 75% patients with disease progression from NAFL to NASH in 44% patients. Among those patients with simple steatosis fibrosis progression was observed in follow up liver biopsy which was statistically more in diabetic steatosis thereby suggesting that simple steatosis can progress to clinically significant fibrosis.[23] Similar data from west suggests that among those with NASH only 21–26% progress to cirrhosis over 8.2 years.[24] Meta-analysis have demonstrated that NAFLD increases the risk of all-cause mortality.[25] Therefore, aggressive management in the form of dietary and lifestyle modification is required in patients who has NASH when compared to those who have simple steatosis. The riddle of progression from NAFL to NASH and subsequently to cirrhosis is poorly understood. In this review, we present new insights into progression of NAFL to NASH, integrating the role of genes, diet, immunological profile, cytokines, liver cell types, and gut microbiota.

JOURNEY FROM NONALCOHOLIC FATTY LIVER DISEASE TO NONALCOHOLIC STEATOHEPATITIS

The spectrum of NAFLD ranges from simple steatosis to NASH. The accumulation of fat above the physiological level (<5%) in the hepatocytes (steatosis) is the prerequisite for development of NASH. Although NAFL is almost universally present in obese individuals yet not all of them progress to NASH. Similarly there are many individuals who may be non-obese physically but may be metabolically obese and have NASH. This indicates that multiple factors other than obesity and insulin resistance are involved in the disease progression. The original "two-hit hypothesis" considered steatosis to be the "first hit", which increased the sensitivity of the liver to the "second hits" that leads to hepatocyte injury and ultimately inflammation (NASH) and fibrosis.[26] However, emerging evidence suggests that the pathogenesis involves "multiple hits"[27] which progress parallel to each other and progression to NASH depends on the close interaction and cross talk between host genes, environmental influences, gut microbiota and host immune system.[28] The progression from NAFL to NASH is poorly understood. The following sections will elaborate the various factors implicated in disease progression (Figure 1).

GENES IN NONALCOHOLIC FATTY LIVER DISEASE

Considerable variation in severity of the disease and rate of progression suggests a genetic predisposition for the disease. It is a polygenic disease with involvement of multiple loci, environmental and nutrient interactions. Furthermore, in a substantial percentage (25–35%) of individuals, the disease is thought to be contributed by a genetic component.[29] Common genetic variants at various loci have consistently been associated with fatty infiltration. Studies have shown that Asian Indians have a genetic predisposition to have up to twofold increased hepatic fat accumulation despite having a low BMI due to their tendency to have more visceral adiposity.[15] The major locus among these is 22q13.31 which harbors the PNPLA3 gene. A particular single nucleotide polymorphism (SNP) namely rs738409 showed strongest association with NAFLD across various Genome wide association studies (GWAS) in various populations[30–32] and this is probably one of the very few SNPs that were identified by GWAS which was significant across various ethnicities. The SNP is located in the 3rd exon of the gene and is mainly expressed by liver and adipose tissue. The gene is involved in triglyceride hydrolysis activity and the SNP introduces an amino acid substitution from isoleucine to methionine (I148M) that abolishes the function.[33] PNPLA-I148M polymorphism has been shown to be associated with increased necroinflammation, severe steatohepatitis and advanced fibrosis.[34] In India, higher frequency of C/G and G/G genotypes of the rs738409 polymorphism was noted in NAFLD subjects and this was associated with significantly higher fasting insulin, HOMA-IR, alanine transaminase and aspartate transaminase levels.[35] In addition to predisposing patients to severe disease,

