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GENETIC BACKGROUND ASSOCIATED WITH THE PROGRESSION OF NON- ALCOHOLIC FATTY LIVER DISEASE TO LIVER FIBROSIS

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ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent cause of chronic liver disease around the world. Individuals with obesity, diabetes, hyperlipidemia, metabolic syndrome, and insulin resistance are more likely to develop steatosis; however, it seldom progresses to steatohepatitis, fibrosis, and cirrhosis. In addition to the environmental factors, genetic variations further modify NAFLD progression. Several epidemiological, familial, and twin studies have elucidated the role of heritability. More recent genome-wide association studies and candidate gene studies have reported certain genes that play a key role in the susceptibility and advancement of NAFLD. The genetic basis of the disease further enhances the understanding of the pathogenic mechanism, which helps in developing novel biomarkers and therapeutic strategies. This review provides a summary of the genes involved in the susceptibility of NAFLD.

INTRODUCTION: In the development of non-alcoholic fatty liver disease NAFLD, a range of factors influence its progression. Some of the factors involved are: environmental factors (diet and sedentary lifestyle), genetic susceptibility, heredity - its involvement has been suggested by various epidemiological, familial, twin studies and case series, race, and ethnicity [1]. NAFLD is more prevalent in the East Asian Indian population, followed by Hispanics, Asians, Caucasians, and African Americans [2, 3]. In addition to the metabolic risk factors, genetic susceptibility in the pathogenesis of metabolic syndrome also impacts NAFLD progression.

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In the past decade, interesting data has been put forth, indicating a strong involvement of genetics in NAFLD. For easier understanding, genetic studies can be divided into two branches: candidate gene studies and genome-wide association studies (GWAS). A candidate gene study analyses the results of previous genomic/proteomic and animal studies. A gene is then selected to investigate its presumed role in the pathogenesis of the disease. Genome-wide association study tests the association between the common traits found in the human genome with that of its polymorphic traits such as disease progression, drug response, etc. Statistical bias can be a roadblock in GWAS [4].

Genome-Wide Association Studies on NAFLD Development And Progression: The first GWAS for non-alcoholic steatohepatitis (NASH) was conducted by Romeo *et al.*, comprised of 9229 single nucleotide polymorphisms (SNPs) from a mixed population that included Hispanic, African American, and European ancestry derived from the

Dallas Heart Study [5, 6]. Hepatic steatosis was evaluated by the non-invasive proton magnetic resonance spectroscopy (1H-MRS) technique [7]. The result was path-breaking as it inferred PNPLA3 variants as strong modifiers of NASH, especially rs738409[G]; I148M- that was entailed with higher hepatic triglyceride content and hepatic inflammation in all ethnic groups but most frequently in Hispanics. The hepatic triglyceride content was found to be twice in PNPLA3 homozygous individuals than in its non-carriers. The homozygous carriers had twice serum aspartate transaminase (AST) and alanine transaminase (ALT) levels. On the other hand, variation in rs6006460[T] was correlated with lower hepatic fat [5, 8].

The association of PNPLA3 in NAFLD has been observed in different Asian ethnicities such as as-Japanese [9], Indian [10,] and Chinese [11]. There were elevated levels of serum ALT in both histologically established NAFLD and NASH [9, 10]. A meta-analysis which aimed to estimate the strength of the effect of I148M (rs738409 C/G) PNPLA3 on NAFLD and its severity among the different population has reported a strong influence of rs738409 on both liver fat accumulation and severity of the disease [12]. A more recent meta-analysis that included 23 case-control studies (6071 NAFLD and 10366 controls) has revealed a significant association of the variant rs738409 with NAFLD and NASH, irrespective of the subject's ethnicities and age [13].

In the past decade, various studies involving PNPLA3 rs738409 variant have revealed its prominent role in hepatic fat content. More recent studies have indicated its role in the portal and lobular inflammation, Mallory-Denk bodies and NASH progression, and advanced fibrosis [14].

Chalasni *et al.* conducted the second GWAS study in 236 non-Hispanic Caucasian women with NAFLD. This study included clinical, laboratory, and histological data and analyzed 324,623SNPs from the 22 autosomal chromosomes. The inferences were drawn after age, body mass index (BMI), diabetes, waist:hipratios, and levels of glycated hemoglobin (HbA1c) were adjusted in multivariate logistic models. Though this study has reported any relationship between PNLPA3 and NASH progression, but the potential markers identified needs to be validated in larger cohort studies. The NAFLD activity score was found to be associated with SNP rs2645424 on chromosome 8 in the gene encoding farnesyl diphosphate farnesyl transferase 1 (FDFT1) (p=6.8×10−7), an enzyme that plays a role in cholesterol biosynthesis. In addition to SNP rs2645424, SNP rs1227756 on chromosome 10 in *COL13A1* ($P=2.0\times10^{-7}$), rs6591182 on chromosome 11 ($P=8.6\times10^{-7}$), and rs887304 on chromosome 12 in *EFCAB4B* ($P=7.7\times10^{-7}$) were associated with hepatic lobular inflammation. rs2499604 on chromosome 1 ($P=2.2\times10^{-6}$), rs6487679 on chromosome 12 in *PZP* ($P=1.3\times10^{-6}$), rs1421201 on chromosome 18 ($P=1.0\times10^{-5}$), and rs2710833 on chromosome 4 ($P=6.3\times10^{-7}$) were associated with increased serum alanine aminotransferase levels. No significant association was established between genetic markers and phenotypic features such as steatosis, ballooning degeneration, portal inflammation, or other features of NAFLD.[15] It is noteworthy to mention that a cohort study carried out in Britain with a sample size of 340 NAFLD patients showed no significant role of FDFT1 rs2645424 SNP in determining the severity of steatosis and fibrosis [16].

Spelioetes *et al.* carried out a meta-analysis of combined GWAS studies datasets. Computed Tomography (CT) was used to analyze the population-based samples for hepatic steatosis. Primarily, the study confirmed the SNP rs738409 of PNPLA3 presence with the NAFLD susceptibility [17]. Furthermore, the study has identified four additional SNPs that were localized in or near the following genes-

Neurocan (NCAN - rs2228603): In a replication study, NCAN was found to be related to histologically established hepatic steatosis. NCAN plays a role in cell adhesion mechanisms and lipoprotein metabolism. Its locus is closely associated with the TM6SF2 minor allele [18].

Protein Phosphatase 1, Regulatory (Inhibitor) Subunit 3B (PPP1R3B - Rs4240624): The encoded protein is involved in regulating glycogen synthesis in liver and skeletal muscle tissue. Alteration in variants near it affects glycemic levels.

Glucokinase Regulator (GCKR - rs780094): This SNP encodes glucose metabolism regulator in the liver. Its alteration affects glucokinase activity, eventually leading to elevated hepatic glycolytic flux, *de novo* lipogenesis, and triglyceride levels. Many studies have confirmed a connection between NAFLD susceptibility, progression to NASH, and GCKR - rs780094 [19]. A study which included both Asian and non-Asian population has also reported similar results [20].

Lysophospholipase-like 1 (LYPLAL1 - rs12137 855): This SNP provides a complementary function to the PNPLA3 in triglyceride metabolism.

The presence of PPP1R3B - Rs4240624 along with PNPLA3 results in increased CT assessed hepatic steatosis but not histologically assessed NAFLD. On the contrary, the rest of the three SNPs, i.e., NCAN, GCKR, and LYPLAL1 together with PNPLA3 resulted not only in increased CT assessed hepatic steatosis but also histologically assessed NAFLD [21].

Following **Table 1** summarizes the effects of genes that were found to be involved in the disease progression in GWAS studies.

TABLE 1: GENES AND SNPS CONFIRMED BY GWAS

GWAS	Author	Gene	SNPs	Effect on liver
First	Romeo *et al.*	PNPLA3 (Patatin-like phospholipase domain-containing 3)	Rs738409[G]	Increases triglyceride levels
Second	Chalasni *et al.*	FDFT1 (Farnesyl diphosphate farnesyl transferase 1)	Rs2645424	Hepatic lobular inflammation
		COL13A1 (Collagen, type XIII, alpha 1)	Rs1227756 Rs659I182	Hepatic lobular inflammation
		EFCAB4B (EF-hand calcium binding domain 4B)	Rs887304	Hepatic lobular inflammation
		PZP (Pregnancy zone protein)	Rs249604 Rs6487679 Rs1421201 Rs2710833	Increases ALT
Third	Speliotes *et al.*	PNPLA3 (Patatin-like phospholipase domain-containing 3)	Rs738409	Increases ALT/AST
		NCAN (Neurocan)	Rs2228603	Alters lipoprotein metabolism
		PPP1R3B (Protein Phosphatase 1, Regulatory (Inhibitor) Subunit 3B)	Rs4240624	Alters glycemic levels
		GCKR (Glucokinase regulator)	Rs780094	Increases hepatic glycotic flux
		LYPLAL1 (Lysophospholipase-like 1)	Rs12137855	Increases triglyceride levels

Potential Candidate Genes Influencing NAFLD/ NASH:

Genetic Modifiers Influencing Risk for Metabolic Syndromes: Certain gene modifies the etiological conditions known to be involved in the disease progression such as obesity and insulin resistance. Apart from the environmental factors, these are affected by the genetic makeup of an individual as well.

Obesity: A cohort of obese patients included in a GWAS has identified the FTO gene, a novel gene candidate. To date, its role in fatty liver disease has not been confirmed, but some studies have used targeted and random mutagenesis techniques which have indicated insights in the pathogenic mechanism [22]. Some studies have also explored the epigenetic imprinting role in obese patients with NAFLD [23].

Insulin Resistance: Various GWAS analysis has identified and confirmed multiple loci for type 2 diabetes mellitus (T2DM) and insulin resistance but very few were identified for NAFLD. Alterations in ectoenzyme nucleotide pyrophosphate phospho-diesterase 1(ENPP1/PC-1), and insulin receptor substrate-1 (IRS-1) influences NAFLD. A study involving 702 NAFLD cases and 310 healthy controls examined the effect of functional ENPP1, PC-1 and IRS-1polymorphisms influencing insulin receptor activity on liver function in NAFLD and the hepatic manifestation of the metabolic syndrome, reported that the ENPP1 121Gln and IRS-1 972Arg polymorphisms were detected in 28.7% and 18.1% of patients and associated with increased body weight/dyslipidemia and diabetes risk, respectively with the fibrosis score of >1 [24]. A smaller study has failed to replicate the results [25].

Genetic Variants Influencing Steatohepatitis / Triglycerides Accumulation in the Liver:

Patatin-like Phospholipase Domain-Containing 3 (PNPLA3): PNPLA3 gene has been identified, validated, and concluded to be associated with NAFLD in varied groups of populations and is considered as a major risk factor in NAFLD susceptibility [5]. Adiponutrin encodes for trans-membrane polypeptide chain that is expressed on endoplasmic reticulum and the lipid membrane of hepatocytes and adipose tissue. The polypeptide chain exhibits triglyceride hydrolase activity [26]. Certain animal and human studies have demonstrated that PNPLA3 gene activity is regulated by glucose and insulin by a mechanism involved in sterol regulatory element-binding protein-1c [27]. PNPPLA3 variant sensitizes the liver and makes it prone to environmental factors. An SNP named I148M variant was found with a risk allele in European and Indian-Asian descentpopulation- it deteriorates the phospholipase activity leading to decreased lipid catabolism and increased phosphatidic acid. Furthermore, a link was found between loss of lipase activity in hepatic stellate cells and a PNPLA3 variant [28]. These studies indicate a relationship between the PNPLA3 gene and its involvement in liver damage. Its involvement has been observed independently in cohorts of both adults and children and is known to phenotypically increase the serum AST/ALT levels [29]. Not only in NAFLD, but I148M have also been associated with an elevated risk of advanced fibrosis and cirrhosis in chronic alcoholics [27].

Microsomal Triglyceride Transfer Protein (MTTP): In liver and intestines, microsomal triglyceride transfer protein (MTTP) is involved in the synthesis and secretion of very-low-density lipoprotein (VLDL). A frame-shift mutation in MTTP leading to loss of its function causes Bassen-Kornzweig syndrome, a rare autosomal recessive disorder wherein the triglycerides are accumulated in hepatocytes [30]. In the promoter region, a transversion from guanine to thiamine has been linked to decreased transcription, lower levels of MTTP, and hepatic failure in triglycerides excretion. A cohort study of 63 patients has suggested that NAFLD patients with low activity homozygous Gallelehave increased the risk of steatohepatitis and higher NASH grade histologically. This data needs to be validated by larger studies. Another study from Brazil which involved biopsy-proven NASH, has failed to replicate the results [31].

Phosphatidylethanolamine Methyltransferase (PEMT): Phosphatidylcholine is involved in VLDL synthesis, and PEMT catalyzes phosphatidylcholine synthesis. A transversion in the non-synonymous region of exon 8 from guanine to adenine has been linked to NAFLD. A study from Japan that included 107 biopsy-proven NASH patients has indicated the V175M amino acid loss of function to be more frequent than that of controls. But these V175M positive cases had lower BMI suggesting an underlying genetic predisposition [32]. Furthermore, another biopsy-based study has concluded similar results, i.e., the positive association of V175M variant with NASH [33].

Apolipoprotein C3 (ApoC3) and Apolipoprotein E: ApoC3 protein expressed in the liver inhibits the lipoprotein lipase (LPL) activity that hydrolyzes the triglycerides into VLDL and chylomicrons inside the plasma. Hypertriglyceridemia can occur due to its overexpression, and hypo-triglyceridaemia occurs due to its absence. Two SNPs, rs2854116 (T-455C) and rs2854117 (C-482T), present in the promoter region of apolipoprotein C3 have been linked to the severity of steatosis in cohorts of Asian Indians and non-Asian descent. Both above-mentioned SNPs in ApoC3 were identified as mediators in postprandial triglyceridaemia by hindering the lipoprotein lipase activity in human and animal studies. The hepatic receptor scavengers engulf the increased chylomicron load, thereby leading to steatosis [34]. It is noteworthy to mention that a study including about 1228 African Americans, 843 European Americans, and 426 Hispanics reported that there is no significant causal relationship between these two SNPs and hepatic triglyceride content or insulin resistance [35]. Another study that included biopsy-proven NAFLD patients have confirmed the dissociation between these two SNPs and the degree of severity of steatosis or fibrosis or NASH [36]. Other studies conducted on Italian [36], British [36], American [35], and Chinese Han [37] subjects have not confirmed the positive association of the ApoC3 SNPs in NAFLD. A meta-analysis has concluded the absence of a significant association of the ApoC3

SNPs in the pathogenesis of NAFLD [38]. The disparity of the data available questions the methodology and the quality of some of these studies.

Apolipoprotein E plays a crucial role in the metabolism of triglycerides. It helps in the removal of circulating chylomicrons and VLDL by enhancing its preferential uptake in LDL receptors [39]. Two SNPs have been identified, each with a different allele (e2, e3, e4) and a total of six ApoE genotypes [40]. The studies conducted on ApoE SNPs have presented conflicting results. A study conducted on the Turkish population reported the association of ApoE 3/3 genotype with an elevated risk of NAFLD and ApoE3/4 genotype showed a protective effect [41]. Interestingly, while Lee *et al*. reported the distribution of ApoE genotype to have no significant difference in NAFLD patients and healthy controls in the Korean population [42]. Yang *et al.* have shown the protective effect of the e4 allele in fatty liver disease [43]. Further-more, e4 carriers have been shown to reduce the risk of NAFLD twice compared to the e3 homozygous carriers [44].

TABLE 2: GENES ALTERED IN DIFFERENT DISEASE CONDITIONS THAT EFFECT THE NAFLD PROGRESSION

Disease condition	Candidate genes identified	Effect on NAFLD progression
Obesity	FTO	Increases the risk of disease progression
Insulin Resistance	• ENPP1 (ectoenzyme nucleotide pyrophosphate phosphodiesterase 1), • IRS-1 (insulin receptor substrate-1)	Increases the risk of disease progression
Steatohepatitis	• PNPLA3 (Patatin-like phospholipase domain-containing 3), • MTTP (Microsomal triglyceride transfer protein), • PEMT (Phosphatidylethanolamine methyltransferase), • Apo C3 (Apolipoprorein C3)	Increases the risk of disease progression
	• Apo E (Apolipoprotein E)	Protective effect
Oxidative Stress	• TNF-α (Tumor Necrosis Factor-α), • IL-1β (Interlukine-1β), • IL-1α (Interlukine-1α), • IL-28B (Interlukine-28B), • MnSOD (Manganese Superoxide Dismutase), • MTHFR (MethylenetetrahydrofolateReductase**)**	Increases the risk of disease progression
	• TLR4 (Toll-Like Receptor 4), • KLF-6 (Kruppel-Like Factor 6), • UCP (Uncoupling protein 2/3)	Protective effect

Genetic Variants Influencing Oxidative Stress in NAFLD/NASH:

Tumor Necrosis Factor-α: Tumor necrosis factor-α (TNF-α) is a crucial pro-inflammatory cytokine having a wide range of functions, including metabolic regulation, auto-immunity, and in the presence of oxidative stress causes cell apoptosis. TNF-α levels are increased in the NASH patients and also has been linked to the development of NAFLD. Higher TNF-α levels correspond to the insulin resistance and activate the inflammatory mediators leading to elevated risk for NASH [45]. TNF2 allele (at position -308) and TNFA allele (at position -238) present in the promoter region is related to an increased susceptibility of NAFLD.A study that was conducted on the Italian population has found a higher prevalence of polymorphism at 238 in the promoter region of TNF-α than that of -308 in NAFLD patients. Polymorphism at TNF-α 238 has also been indicated not only in impaired insulin sensitivity but also a lower level of LDL-cholesterol and lower BMI suggesting the alterations in the pathways of glucose and lipid metabolism [46].

Polymorphism at TNF-α 1031 and -863 in the promoter region was found more commonly in NASH patients than in simple steatosis in the Japanese population. Interestingly, Japanese patients failed to show polymorphism at -238 and -308 positions due to the lower recurrence of these variants in this ethnicity. A meta-analysis has identified TNF-α 238 as the susceptibility factor in NAFLD but not the TNF-α 308 [47].

Interleukin (IL)-6 and IL- 1β: Interleukin-6 (IL-6) is another cytokine that is involved in both inflammation and insulin resistance. It is produced by hepatic cells, adipocytes, and immune cells. Its serum levels are elevated in people with type 2 diabetes and obesity [48]. Two variants of IL-6 have been identified: IL-6 −174C and IL-6-174G [34]. The IL-6 174C variant has been linked to insulin resistance, T2D, and MS [48, 49] in an Italian cohort with NAFLD [25]. In contrast, certain studies have reported the presence of the IL-6 174G variant to be related to metabolic syndromes [50].

Studies have suggested that IL-1α and IL-1β are involved in the progression of steatosis to steatohepatitis and ultimately liver fibrosis [51]. Polymorphism of IL-1β-511 T/C genotypes that affects the *in-vitro* protein interactions was reported by a Japanese study where NASH cases were compared to 100 healthy controls. This study has suggested that the serum levels of IL-1β were significantly elevated in NASH cases [52]. Furthermore, the number of IL-1β-511 T SNP and T/T genotypes was greater in NASH patients.

Toll-Like Receptor 4: Toll-like receptor 4 (TLR 4) is a transmembrane receptor that plays a vital role in cellular apoptosis and is involved in the activation of Kupffer cells in NAFLD. Recently, bacterial growth and endotoxemia have been reported to be involved in NASH pathogenesis [53]. Disruption in its hepatic signaling can cause hepatic inflammation and injury in NAFLD [54].

TLR4-D299G and T3991 variants have been indicated to decrease the response towards endotoxins, thus suggesting to have an effect on insulin resistance, T2D, and MS [54]. A study by Guo *et al*., has reported that D299G and T3991 have a protective function in the progression of fibrosis.[55] Animal studies have suggested the relationship between TLR4 and Kupffer cells in NASH pathogenesis [53]. A case-control study has shown a decreased number of mutations at -299 in NAFLD cases than that in controls [56]. Though animal and human studies have suggested a protective role of TLR4 in NAFLD/NASH, more studies are required to provide strong evidence.

IL- 28B: Various studies have reported genetic variants in IL-28B in removing hepatic C infection. IL-28B- rs12979860 CC and rs8099917 TT variants are associated with the consistent viral response after anti-viral therapy [57]. Some studies have suggested that IL-28B polymorphism can be attributed to the degree of severity of inflammation and progression to fibrosis [58]. A study with 160 NAFLD cases has identified IL-28 rs 12979860 CC in the severity of the hepatic diseases [59].

As the IL-28 rs12979860 CC variant was frequently observed in patients with the PNPLA3 G allele, a hypothesis was assumed regarding their inter-dependence. Eslam *et al*., have tested and confirmed this hypothesis on a larger cohort with 3129 CHC, 555 hepatitis B, and 488 NAFLD patients. They have reported that the presence of rs`12979860 SNP can be used as a genetic marker in inflammation and fibrosis in chronic hepatic diseases [60]. A North American study conducted on the Caucasian population with NAFLD did not reveal any association between the SNP and inflammation [61].

Superoxide Dismutase 2 and Cytochrome P450 2E1: MnSOD plays a vital role in protecting the hepatic cells from the mitochondrial superoxide radicals. MnSOD is encoded by superoxide dismutase 2 (SOD 2) gene.[62] C47T rs4880 SNP in SOD 2 gene has been linked to the disruption in the signal sequence that leads to the substitution of Ala16Val [63]. The Ala allele causes relatively increased protein import leading to elevated enzymatic activity. A Japanese study that included 63 subjects has demonstrated the decreased enzyme activity in homozygous T genotype patients [64]. An Egyptian study has revealed similar results in obese children with hepatic steatosis and NASH [65]. Al Serri *et al*., have reported the association of C4TT and fibrosis, suggesting that oxidative stress is a crucial factor in the pathogenesis of NAFLD [66].

Uncoupling Protein 3 and Uncoupling Protein 2: Uncoupling protein 3 (UCP 3) is a mitochondrial transporter that increases the proton leak from the inner mitochondrial membrane and catalyzes the oxidative phosphorylation. It regulates the body's energy modulation and lipid homeostasis. It is usually expressed in skeletal muscle [67]. The polymorphism in the promoter region of UCP 3 *i.e*., rs1800849-55 C/T variants, has been associated with greater susceptibility to T2D and

obesity in atherosclerotic patients [68]. A Spanish study has linked the CT genotype to IR, the higher adiponectin levels, the presence of steatosis and NASH [69].

Some studies have reported the protective role of the increased hepatic expression of uncoupling protein 2 (UCP-2) in NASH. An SNP in the promoter region of-2, -866 G>A variant affects the extra-hepatic expression of UCP-2 and insulin release and sensitivity [70]. A more recent study has suggested the role of -866 A/A genotype in decreasing the progression of NASH after the confounding factors were adjusted [71]. Further studies are needed to establish the protective or the susceptibility role of UCP in NAFLD/NASH.

Methylenetetrahydrofolate Reductase: Hyper-homocysteinaemia is considered a risk factor in liver diseases. Any disruption in the metabolic pathway of cysteine due to genetic polymorphism affects liver damage. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the methylation of homocysteine [72]. Sazci *et al*., have reported MTHFR 1298C in NASH, C677C/C1298C variants in women, and C677C/A12988C in men increases the susceptibility to NASH progression [73]. Interestingly, other studies have failed to replicate similar results. Thus, its definite role in NAFLD/NASH cannot be stated [74].

Kruppel-Like Factor 6: Liver injury leads to the cytokine release that increases the Kruppel-like factor 6 (KLF-6) gene expression that plays a vital role in the activation of other genes involved in the progression of liver fibrosis [75]. A study has reported the relationship between the polymorphism in KLF-6 gene IVS1-27G>A SNP (rs3750861) and the severity of NAFLD. The levels of total and wild-type KLF-6 expression are increased in NAFLD and steatosis. On the other hand, KLF-6 IVS1-27G>A SNP has been linked to the protective action against the progression of NASH [76].

CONCLUSION: NAFLD is a complex disease, and its development and progression are influenced by various genetic and environmental factors. Recent findings from GWAS and candidate gene studies have confirmed various genetic variants that are involved in the progression of NASH. This has further enhanced the understanding of the rapid advancement of NAFLD to NASH. Genetic assessment of the patients for the risk factors can be used as a tool that will help provide tailored and targeted therapy to halt the progression to NASH. The greater challenge is to translate the newer genetic findings into therapeutic benefits.

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REFERENCES:

1. Anstee QM, Daly AK and Day CP: Genetics of alcoholic and nonalcoholic fatty liver disease. Semin Liver Dis 2011; 31(2): 128-46.
2. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J and Dalla Man C: Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. Proc Natl Acad Sci U S A. 2006; 103(48): 18273-7.
3. Schneider AL, Lazo M, Selvin E and Clark JM: Racial differences in nonalcoholic fatty liver disease in the US population. Obesity 2014; 22(1): 292-9.
4. Lewis CM and Knight J: Introduction to genetic association studies. Cold Spring Harb Protoc 2012; 2012(3): 297-306.
5. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D and Pennacchio LA: Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008; 40(12): 1461-5.
6. Victor RG, Haley RW, Willett DL, Peshock RM, Vaeth PC and Leonard D: The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. Am J Cardiol 2004; 93(12): 1473-80.
7. Siegelman ES and Rosen MA: Imaging of hepatic steatosis. Semin Liver Dis 2001; 21(1): 71-80.
8. Romeo S, Huang-Doran I, Baroni MG and Kotronen A: Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. Curr Opin Lipidol 2010; 21(3): 247-52.
9. Akuta N, Kawamura Y, Arase Y, Suzuki F, Sezaki H and Hosaka T: Relationships between genetic variations of PNPLA3, TM6SF2 and histological features of nonalcoholic fatty liver disease in Japan. Gut Liver 2016; 10(3): 437-45.
10. Kanth VV, Sasikala M, Rao PN, Avanthi US, Rao KR and Reddy DN: Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study. World J Hepatol 2014; 6(6): 435-42.
11. Zhang Y, Cai W, Song J, Miao L, Zhang B and Xu Q: Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uygur and Han ethnic groups of northwestern China. PLoS One 2014; 9(10): e108381.
12. Singal AG, Manjunath H, Yopp AC, Beg MS, Marrero JA and Gopal P: The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. Am J Gastroenterol 2014; 109(3): 325-34.
13. Xu R, Tao A, Zhang S, Deng Y and Chen G: Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. Sci Rep 2015; 5: 9284.

14. Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R and Dongiovanni P: Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010; 51(4): 1209-17.
15. Chalasani N, Guo X, Loomba R, Goodarzi MO, Haritunians T and Kwon S: Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. Gastroenterology 2010; 139(5): 1567-76.
16. Ballestri S, Day CP and Daly AK: Polymorphism in the farnesyl diphosphate farnesyl transferase 1 gene and nonalcoholic fatty liver disease severity. Gastroenterology 2011; 140(5): 1694-5.
17. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ and Palmer CD: Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011; 7(3): e1001324.
18. Gorden A, Yang R, Yerges-Armstrong LM, Ryan KA, Speliotes E and Borecki IB: Genetic variation at NCAN locus is associated with inflammation and fibrosis in non-alcoholic fatty liver disease in morbid obesity. Hum Hered 2013; 75(1): 34-43.
19. Tan HL, Zain SM, Mohamed R, Rampal S, Chin KF and Basu RC: Association of glucokinase regulatory gene polymorphisms with risk and severity of non-alcoholic fatty liver disease: an interaction study with adiponutrin gene. J Gastroenterol 2014; 49(6): 1056-64.
20. Zain SM, Mohamed Z and Mohamed R: A common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: A meta-analysis. J Gastroenterol Hepatol 2015; 30(1): 21-7.
21. Anstee QM and Goldin RD: Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. Int J Exp Pathol 2006; 87(1): 1-16.
22. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC and Rüther U: Inactivation of the Fto gene protects from obesity. Nature 2009; 458(7240): 894-8.
23. Kelly ML, Moir L, Jones L, Whitehill E, Anstee QM and Goldin RD: A missense mutation in the non-neural G-protein α-subunit isoforms modulates susceptibility to obesity. Int J Obes (Lond) 2009; 33(5): 507-18.
24. Dongiovanni P, Valenti L, Rametta R, Daly AK, Nobili V and Mozzi E: Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. Gut 2010; 59(2): 267-73.
25. Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D and Fargion S: Genetic polymorphisms in non-alcoholic fatty liver disease: Interleukin-6− 174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis 2009; 41(11): 823-8.
26. Pingitore P, Pirazzi C, Mancina RM, Motta BM, Indiveri C and Pujia A: Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. BiochimBiophys Acta 2014; 1841(4): 574-80.
27. Dubuquoy C, Robichon C, Lasnier F, Langlois C, Dugail I and Foufelle F: Distinct regulation of adiponutrin/PNPLA3 gene expression by the transcription factors ChREBP and SREBP1c in mouse and human hepatocytes. J Hepatol 2011; 55(1): 145-53.
28. Yuan X, Waterworth D, Perry JR, Lim N, Song K and Chambers JC: Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. Am J Hum Genet 2008; 83(4): 520-8.
29. Kollerits B, Coassin S, Kiechl S, Hunt SC, Paulweber B and Willeit J: A common variant in the adiponutrin gene influences liver enzyme values. J Med Genet 2010; 47(2): 116-19.
30. Lonardo A, Lombardini S, Scaglioni F, Carulli L, Ricchi M and Ganazzi D: Hepatic steatosis and insulin resistance: does etiology make a difference? J Hepatol 2006; 44(1): 190-6.
31. Oliveira CP, Stefano JT, Cavaleiro AM, Zanella Fortes MA, Vieira SM and Rodrigues-Lima VM: Association of polymorphisms of glutamate-cystein ligase and microsomal triglyceride transfer protein genes in non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2010; 25(2): 357-61.
32. Dong H, Wang J, Li C, Hirose A, Nozaki Y and Takahashi M: The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. J Hepatol 2007; 46(5): 915-20.
33. Song J, da Costa KA, Fischer LM, Kohlmeier M, Kwock L and Wang S: Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). FASEB J 2005; 19(10): 1266-71.
34. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN and Zhang XM: Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 2010; 362(12): 1082-9.
35. Kozlitina J, Boerwinkle E, Cohen JC and Hobbs HH: Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. Hepatology 2011; 53(2): 467-74.
36. Valenti L, Nobili V, Al-Serri A, Rametta R, Leathart JB and Zappa MA: The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. J Hepatol 2011; 55(6): 1409-14.
37. Niu TH, Jiang M, Xin YN, Jiang XJ, Lin ZH and Xuan SY: Lack of association between apolipoprotein C3 gene polymorphisms and risk of nonalcoholic fatty liver disease in a Chinese Han population. World J Gastroenterol 2014; 20(13): 3655-62.
38. Zhang H, Chen L, Xin Y, Lou Y, Liu Y and Xuan S: Apolipoprotein c3 gene polymorphisms are not a risk factor for developing non-alcoholic fatty liver disease: a meta-analysis. Hepat Mon 2014; 14(10).
39. Karavia EA, Papachristou DJ, Kotsikogianni I, Giopanou I and Kypreos KE: Deficiency in apolipoprotein E has a protective effect on diet-induced nonalcoholic fatty liver disease in mice. FEBS J 2011; 278(17): 3119-29.
40. Mahley RW, Nathan BP and Pitas RE: Apolipoprotein E. Structure, Function, and Possible Roles in Alzheimer's Disease a. Ann N Y Acad Sci 1996; 777(1): 139-45.
41. Sazci A, Akpinar G, Aygun C, Ergul E, Senturk O and Hulagu S: Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. Dig Dis Sci 2008; 53(12): 3218-24.
42. Lee DM, Lee SO, Mun BS, Ahn HS, Park HY and Lee HS: Relation of apolipoprotein E polymorphism to clinically diagnosed fatty liver disease. Taehan Kan Hakhoe Chi 2002; 8(4): 355-62.
43. Yang MH, Son HJ, Sung JD, Choi YH, Koh KC and Yoo BC: The relationship between apolipoprotein E polymorphism, lipoprotein (a) and fatty liver disease. Hepatogastroenterology 2005; 52(66): 1832-5.
44. De Feo E, Cefalo C, Arzani D, Amore R, Landolfi R and Grieco A: A case–control study on the effects of the

apolipoprotein E genotypes in nonalcoholic fatty liver disease. Mol Biol Rep 2012; 39(7): 7381-8.
45. Wong VW, Hui AY, Tsang SW, Chan JL, Tse AM and Chan KF: Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2006; 4(9): 1154-61.
46. Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A and Taioli E: Tumor necrosis factor α promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 2002; 122(2): 274-80.
47. Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E and Shiratori K: Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. J Hepatol 2007; 46(6): 1104-10.
48. Stojsavljević S, Palčić MG, Jukić LV, Duvnjak LS and Duvnjak M: Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroente 2014; 20(48): 18070-91.
49. Berthier MT, Paradis AM, Tchernof A, Bergeron J, Prud'homme D and Després JP: The interleukin 6–174G/C polymorphism is associated with indices of obesity in men. J Hum Genet 2003; 48(1): 14-9.
50. Cardellini M, Perego L, D'Adamo M, Marini MA, Procopio C and Hribal ML: C-174G polymorphism in the promoter of the interleukin-6 gene is associated with insulin resistance. Diabetes Care 2005; 28(8): 2007-12.
51. Kamari Y, Shaish A, Vax E, Shemesh S, Kandel-Kfir M and Arbel Y: Lack of interleukin-1α or interleukin-1β inhibits transformation of steatosis to steatohepatitis and liver fibrosis in hypercholesterolemic mice. J Hepatol 2011; 55(5): 1086-94.
52. Nozaki Y, Saibara T, Nemoto Y, Ono M, Akisawa N and Iwasaki S: Polymorphisms of Interleukin-1β and β3-Adrenergic Receptor in Japanese Patients With Nonalcoholic Steatohepatitis. Alcohol Clin Exp Res 2004; 28: 106S-110S.
53. Rivera CA, Adegboyega P, van Rooijen N, Tagalicud A, Allman M and Wallace M: Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. J Hepatol 2007; 47(4): 571-9.
54. Steinhardt AP, Aranguren F, Tellechea ML, Rosso LA, Brites FD and Martínez-Larrad MT: A functional non-synonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation. Metabolism 2010; 59(5): 711-7.
55. Guo J, Loke J, Zheng F, Hong F, Yea S and Fukata M: Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. Hepatology 2009; 49(3): 960-8.
56. Kiziltas S, Ata P, Colak Y, Mesçi B, Senates E and Enc F: TLR4 gene polymorphism in patients with nonalcoholic fatty liver disease in comparison to healthy controls. Metab Syndr Relat Disord 2014; 12(3): 165-70.
57. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J and Mueller T: Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010; 138(4): 1338-45.
58. Marabita F, Aghemo A, De Nicola S, Rumi MG, Cheroni C and Scavelli R: Genetic variation in the interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. Hepatology 2011; 54(4): 1127-34.
59. Thompson AJ, Clark PJ, Fellay J, Muir AJ, Tillmann HL and Patel K: IL28B genotype is not associated with advanced hepatic fibrosis in chronic hepatitis C patients enrolled in the IDEAL study. Hepatology 2010; 52(4): 437A-8A.
60. Eslam M, Hashem AM, Leung R, Romero-Gomez M, Berg T and Dore GJ: Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. Nat Commun 2015; 6: 6422.
61. Garrett ME, Abdelmalek MF, Ashley-Koch A, Hauser MA, Moylan CA and Pang H: IL28B rs12979860 is not associated with histologic features of NAFLD in a cohort of Caucasian North American patients. J Hepatol 2013; 58: 402-03.
62. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y and Mizuno Y: Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene: a predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun 1996; 226(2): 561-5.
63. Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S and Pessayre D: The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics 2005; 15(5): 311-9.
64. Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y and Ono M: Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol 2004; 40(5): 781-6.
65. El-Koofy NM, El-Karaksy HM, Mandour IM, Anwar GM, El-Raziky MS and El-Hennawy AM: Genetic polymorphisms in non-alcoholic fatty liver disease in obese Egyptian children. Saudi J Gastroenterol 2011; 17(4): 265.
66. Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB and Dongiovanni P: The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. J Hepatol 2012; 56(2): 448-54.
67. De Luis DA, Aller R, Izaola O, Sagrado MG and Conde R: Modulation of adipocytokines response and weight loss secondary to a hypocaloric diet in obese patients by-55CT polymorphism of UCP3 gene. Horm Metab Res 2008; 40(03): 214-8.
68. Schrauwen P, Xia J, Walder K, Snitker S and Ravussin E: A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians. Intl J obesity Relat Metab Disord 1999; 23(12): 1242-5.
69. Aller R, De Luis DA, Izaola O, Sagrado MG, Conde R and Alvarez T: Role of-55CT polymorphism of UCP3 gene on non alcoholic fatty liver disease and insulin resistance in patients with obesity. Nutr Hosp 2010; 25(4): 572-6.
70. Jia JJ, Zhang X, Ge CR and Jois M: The polymorphisms of UCP2 and UCP3 genes associated with fat metabolism, obesity and diabetes. Obes Rev 2009; 10(5): 519-26.
71. Fares R, Petta S, Lombardi R, Grimaudo S, Dongiovanni P and Pipitone R: The UCP 2-866 G> A promoter region polymorphism is associated with nonalcoholic steatohepatitis. Liver Int 2015; 35(5): 1574-80.
72. Ventura P, Rosa MC, Abbati G, Marchini S, Grandone E and Vergura P: Hyperhomocysteinaemia in chronic liver diseases: role of disease stage, vitamin status and methylenetetrahydrofolate reductase genetics. Liver Int 2005; 25(1): 49-56.

73. Sazci A, Ergul E, Aygun C, Akpinar G, Senturk O and Hulagu S: Methylenetetrahydrofolate reductase gene polymorphisms in patients with nonalcoholic steato-hepatitis (NASH). Cell Biochem Funct 2008; 26(3): 291-6.
74. Catalano D, Trovato GM, Ragusa A, Martines GF, Tonzuso A and Pirri C: Non-alcoholic fatty liver disease (NAFLD) and MTHFR 1298A> C gene polymorphism. Eur Rev Med Pharmacol Sci 2014; 18(2): 151-9.
75. Ratziu V, Lalazar A, Wong L, Dang Q, Collins C and Shaulian E: Zf9, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. Proc Natl Acad Sci U S A. 1998; 95(16): 9500-9505.
76. Miele L, Beale G, Patman G, Nobili V, Leathart J and Grieco A: The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. Gastroenterology 2008; 135(1): 282-91.

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