TOPIC HIGHLIGHT

2016 Nonalcoholic Fatty Liver Disease: Global view

Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management

Vishnubhotla Venkata Ravi Kanth, Mitnala Sasikala, Mithun Sharma, Padaki Nagaraja Rao, Duvvuru Nageshwar Reddy

Vishnubhotla Venkata Ravi Kanth, Mitnala Sasikala, Asian Healthcare Foundation, a Research Wing of Asian Institute of Gastroenterology, Hyderabad 500082, Telangana, India

Mithun Sharma, Padaki Nagaraja Rao, Duvvuru Nageshwar Reddy, Asian Institute of Gastroenterology, Hyderabad 500082, Telangana, India

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Correspondence to: Dr. Mitnala Sasikala, Head of Research Labs, Asian Healthcare Foundation, a Research Wing of Asian Institute of Gastroenterology, 6-3-661, Somajiguda, Hyderabad 500082, Telangana, India. aigres.mit@gmail.com
Telephone: +91-40-23378888-701
Fax: +91-40-23324255

Abstract

Genetics plays an important role in determining the susceptibility of an individual to develop a disease. Complex, multi factorial diseases of modern day (diabetes, cardiovascular disease, hypertension and obesity) are a result of disparity between the type of food consumed and genes, suggesting that food which does not match the host genes is probably one of the major reasons for developing life style diseases. Non-alcoholic fatty liver is becoming a global epidemic leading to substantial morbidity. While various genotyping approaches such as whole exome sequencing using next generation sequencers and genome wide association studies have identified susceptibility loci for non-alcoholic fatty liver disease (NAFLD) including variants in patatin-like phospholipase domain containing 3 and transmembrane 6 superfamily member 2 genes apart from others; nutrient based studies emphasized on a combination of vitamin D, E and omega-3 fatty acids to manage fatty liver disease. However majority of the studies were conducted independent of each other and very few studies explored the interactions between the genetic susceptibility and nutrient interactions. Identifying such interactions will aid in optimizing the nutrition tailor made to an individual's genetic makeup, thereby aiding in delaying the onset of the disease and its progression. The present topic focuses on studies that identified the genetic susceptibility for NAFLD, nutritional recommendations, and their interactions for better management of NAFLD.

Key words: Transmembrane 6 superfamily member 2 gene; Patatin-like phospholipase domain containing 3 gene; Genotyping; Nutrient interactions; Non-alcoholic fatty liver disease; Genetic susceptibility

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Core tip: Various genome wide association and replication studies across ethnicities have consistently associated variants in patatin-like phospholipase domain containing 3 gene with a higher risk of non-alcoholic fatty liver disease (NAFLD). More recently a variant in transmembrane 6 superfamily member 2 gene was also associated with susceptibility to the disease. Functional studies have established the role of these genes in NAFLD. Gene and nutrient interactions should be the focus of future research in the management of NAFLD.

INTRODUCTION

Genetic susceptibility carried by an individual determines the risk of developing a disease. However, not all individuals who carry the risk manifest with the disease, suggesting that, most of the complex multifactorial diseases are the result of interactions between genes and environment. Disease occurrence (onset) or severity may differ in individuals with same genotype exposed to different environmental conditions or vice versa, reiterating the fact that phenotype is the consequence of genotype and environment interactions (Figure 1). Diet, life style, exposure to chemicals and toxins form the major part of environmental risks. Majority of the modern day life style diseases such as diabetes, cardiovascular disease, hypertension, obesity are typically inherited with multifactorial mode of inheritance. It refers to a complex pattern of inheritance where a combination of both genetic and other factors including environmental are involved. Multifactorial conditions do not always manifest despite the fact that the individual carries a genetic variant that increases the risk of disease, putting the emphasis on favorable environment.

Non-alcoholic fatty liver disease (NAFLD) is one of the most important lifestyle based complex and multifactorial diseases. The prevalence of the disease varies markedly in various populations. It ranges between 20%-30% in the Western countries[1], 20%-30% in Europeans[2], 8% in Japanese[3] and 25%-30% in Indians[4]. The spectrum of the disease ranges between steatosis alone on one hand and non-alcoholic steatohepatitis (NASH)/cirrhosis/hepatocellular carcinoma on the other. However, the progression through the spectrum involves multiple risks including genetic and environmental interactions, in addition to other risk factors (Obesity, advancing age, diabetes, hypertension, and hypertriglyceridemia).

Therefore, understanding genetic susceptibility has been the major focus of recent research in addition to alterations in dietary habits and life style modifications which have been demonstrated to benefit the patients and aid in better management of the disease.

It is imperative for organisms from bacteria to humans to regulate their metabolism vis a vis availability of nutrients for better survival of the species. Nutrient-gene interactions have therefore been an ancient and omnipresent mechanism across species. However research started to explore these interactions only lately and the topic has been of prime importance in the context of disease including NAFLD. This review focuses on the genetic susceptibility identified till date employing various approaches (Exome sequencing, GWAs, candidate gene) thus far and the nutrient risks and the interactions between the two wherever studies are available.

WHOLE EXOME SEQUENCING AND NAFLD

Recent advances in sequencing the human genome have transformed methods of identifying genetic susceptibility for complex, multifactorial diseases. With whole exome sequencing studies, it is now possible to sequence protein coding regions of the genome and identify genetic susceptibility for complex diseases in an unbiased manner. Although very few studies are available that exploited this technology, important loci have been identified. A quick search on PubMed revealed a single whole exome sequencing study in morbidly obese patients of Caucasian origin with NAFLD, that revealed novel damaging mutations in Bardet-Biedl syndrome 1 gene and Melanocortin 3 receptor gene (*MC3R*). *MC3R* gene encodes MC3 a G-protein coupled receptor for melanocyte stimulating hormone and adenocorticotropic hormone. Studies have identified that mice deficient for this gene product have increased fat and play a critical role in weight regulation ("Entrez gene: *MC3R* melanocortin 3 receptor"). Further another patient with NAFLD-related cirrhosis was compound heterozygous for rare and damaging mutations in patatin-like phospholipase domain containing 3 (PNPLA3)[5]. It is only recently that researchers have started to harness NGS technology to identify genetic susceptibility for complex diseases. In future by exploiting this technology many more important loci may be identified for NAFLD that may aid in better management of the disease.

GENOME WIDE ASSOCIATION STUDIES AND NAFLD

Genome wide association studies (GWAS) are employed to identify genetic susceptibility for complex diseases in an unbiased way. One of the first GWA studies for NAFLD was performed by Romeo *et al*[6] that used a custom chip of approximately 9000 non-synonymous variants across

Table 1 List of genome/exome wide association studies and loci identified for non-alcoholic fatty liver disease

Ref.	Phenotype associated with	Ancestry of samples included	Genotyping platform	Discovery sample size	Replication sample size	Genes
Romeo *et al*[6], 2008	Increased hepatic fat levels and inflammation	Hispanic, African American and European American individuals	Perlegen Sciences Custom array (12138 NS variation)	2111 individuals with MRS measured hepatic steatosis	None	*PNPLA3*
Chalasani *et al*[15], 2010	Features of hepatic histology	Non-Hispanic white women	Illumina (324,623 SNPs)	236, non-Hispanic white women	None	*FDFT1, rs343062 (Chr 7), COL13A1, rs6591182 (Chr 11), EFCAB4B, rs2499604 (chr 1), PZP, rs1421201 (Chr 18) rs2710833 (Chr 4)*
Speliotes *et al*[16], 2011	CT measured hepatic steatosis	European american including Amish	Affymetrix, Illumina	7126 with CT measured hepatic steatosis	592/1405	*PNPLA3, NCAN, PPP1R3B, GCKR, LYPLAL1*
Kawaguchi *et al*[3], 2012	NAFLD	Japanese	Illumina	529 patients consisting of four NAFLD subgroups (Matteoni's classification)	None	*PNPLA3, SAMM50, PARVB, HS3ST1-HSP90AB2P, YIPF1*
Kitamoto *et al*[66], 2013	NAFLD	Japanese	Illumina	392 NAFLD and 934 controls	172 NAFLD and 1012 controls	*PNPLA3, SAMM50, PARVB* gene
Kozlitina *et al*[19], 2014	MRS measured hepatic steatosis	Hispanic, African, American and European	Illumina	2,736	None	*PNPLA3* and *TM6SF2*

NAFLD: Non-alcoholic fatty liver disease; CT: Computed tomography; SNPs: Single nucleotide polymorphisms; MRS: Magnetic resonance spectroscopy.
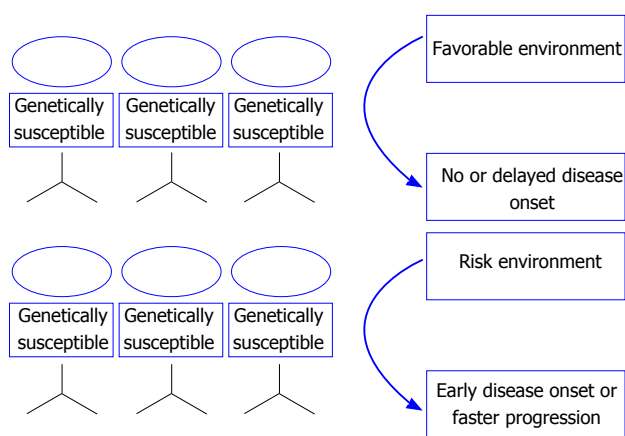

Figure 1 Genetic and environmental interactions to produce a phenotype.

the genome. The sample included patients with and without NAFLD of various ethnicities including European, Hispanic and African-American. The liver fat was measured by proton magnetic resonance spectroscopy. One variant (rs738409), a G allele encoding I148M in *PNPLA3* gene was associated with increased fat level in the liver across all the ethnicities. A list of various GWA studies and the variants identified are given in Table 1. Subsequently various groups have replicated the association of this variant in different ethnicities including Japanese[3,7], Indian[8,9], Chinese[10,11]. Further the variant was also associated with higher levels of ALT, histologic NAFLD including steatosis[7,8].

A meta-analysis of 24 studies that included 9915

patients from different ethnicities, identified that PNPLA3 rs738409 variant was associated with fibrosis severity (OR = 1.32, 95%CI: 1.20-1.45)[12]. Another meta-analysis of 16 studies[13], showed that rs738409 had a strong influence on liver fat accumulation. Individuals with GG homozygous genotype showed 77% higher lipid fat content compared to CC genotype and were susceptible to 3.24 fold aggressive disease and NASH. Further, when the risk associated with heterozygosity was evaluated for the variant, additive genetic model was better at explaining the effect of the variant on the susceptibility to develop NAFLD. However the analysis suggested that carrying two G alleles did not seem to increase the risk of severe histological features. Also, meta-regression showed a negative correlation between male sex and the effect of rs738409 on liver fat content (slope: -2.45 ± 1.04; *P* < 0.02). Importantly, the rs738409 GG genotype *vs* the CC genotype was associated with a 28% increase in serum alanine amino-transferase levels. Xu *et al*[14] recent meta analysis of the rs738409 variant that included 23 case-control studies (6071 NAFLD and 10366 controls) showed a significant association of the variant with NAFLD, NASH. The subgroup and sensitivity analysis revealed that the changes were not influenced by the ethnicities and age of the subjects.

A GWA study conducted[15] in 236 non-Hispanic white woman who were genotyped for 3,24,623 single nucleotide polymorphisms (SNPs) on the Illumina platform and were assessed for various histologic parameters revealed that NAFLD activity score was associated with

rs2645424 in farnesyl diphosphate farnesyl transferase 1. Further analysis revealed that degree of fibrosis was associated with rs343062, lobular inflammation with rs1227756 in COL13A1), rs6591182, and rs887304 in EFCAB4B. SNPs associated with serum levels of alanine aminotransferase included rs2499604, rs6487679, rs1421201 and rs2710833. However, no significant associations were found between genotypes and steatosis, ballooning degeneration, portal inflammation, or other features of NAFLD.

A meta-analysis[16] carried out across four groups of European ancestry and one of the largest GWA studies for NAFLD was tested for associations with computed tomography (CT) measured steatosis initially in the 4 groups independently followed by a meta-analysis. The study involved 7176 individuals that were controlled for age, gender and all the principal components. Variants in or near *PNPLA3, LYPLAL1, PPP1R3B, NCAN/*transmembrane 6 superfamily member 2 (*TM6SF2*) and *GCKR* genes were found to be associated with hepatic steatosis. These above variants but for *PPP1R3B* were also associated with NASH and fibrosis.

Few GWA studies identified loci associated with the associated parameters of NAFLD and most importantly the liver function tests. Two such studies[17,18] have identified four loci namely SNPs in or near *PNPLA3* (rs2281135, rs738409), *SAMM50* (rs2143571, *CPN1-ERLIN1-CHUK* gene cluster (rs10883437, rs11597390, rs11591741, rs11597086), *TRIB1* (rs2954021) and near *HSD17B13/ MAPK10* (rs6834314) that were associated with elevated levels of ALT.

Our pooled genetic study[8], where 19 variants were selected that were associated with NAFLD from 4 GWA studies conducted until 2013 and replicated in patients with and without ultrasound detected NAFLD in Indians. The study identified variants in *PNPLA3, PZP, SAMM50* and *PARVB* were associated with NAFLD. Furthermore, the haplotype data suggested that variants in *PNPLA3, SAMM50* and *PARVB* on chromosome 22 were linked, suggesting that this loci is very important in Indian context. Studies from Japan[3] also associated these loci with NAFLD suggesting that it is an important loci conferring susceptibility in Asian population.

WHOLE EXOME ASSOCIATION STUDY AND NAFLD

Two studies published during the same time reported the association of a variant (rs58542926) in *TM6SF2* gene with susceptibility to NAFLD[19] and influencing total cholesterol and myocardial infarction risk[20]. The first study identified that the variant in *TM6SF2* gene was associated with hepatic triglyceride content (HTGC) and is a adenine to guanine substitution in coding nucleotide 499, replacing glutamate with lysine at position 167 (c.499A > G; p.Glu167Lys). The frequency of this variant was higher in three ancestries studied (European, African-American and Hispanics). The study suggested

that the variant carriers had elevated mean and median HTGC in European and African-American ancestries. The study also identified that there was a reduction in the expression of recombinant protein in cultured hepatocytes by almost 50% by the Glu167Lys *TM6SF2* variant compared to the wild type. Further knockdown of the gene by Adeno-associated virus-mediated short hairpin RNA in mice increased the liver triglyceride content by threefold and decreased very-low-density lipoprotein (VLDL) secretion by half. Based on the above, the study suggested that TM6SF2 activity may be required for normal VLDL secretion and that impaired function of the *TM6SF2* gene causally contributes to NAFLD[19]. Further, the function of the gene was also clearly established, where it is now known to be a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content[21]. The second study[20], systematically assessed coding variants at the genome-wide level to identify novel lipid genes and also evaluate whether low frequency variants with large effect exist, identified a coding variant (p.Glu167Lys) in *TM6SF2* gene that modifies total cholesterol levels and further was associated with myocardial infarction. The functional role of few of the genes identified employing GWAS and exome wide association studies are as given in Table 2.

In an ongoing study at our center, we have replicated these variants (rs58542926 in *TM6SF2* and rs2281135 in *PNPLA3* genes) in 220 patients with NAFLD and 185 controls to date. Both the variants are significantly associated with the disease (*TM6SF2 P* = 0.00008; *PNPLA3 P* = 0.002) with a higher risk of the disease (Odds - 2.17, 95%CI: 1.34-3.52 and 1.85, 95%CI: 1.24-2.76). Further both the variants were significantly associated (*P* > 0.05) with higher ALT and AST levels (data unpublished).

CANDIDATE GENE STUDIES (OTHER GENES) AND NAFLD

Based on the two hit hypothesis as discussed earlier[22], studies have explored genes that have an important role in mechanisms related to lipid metabolism, insulin signaling, oxidative stress, inflammation and fibrogenesis.

While *APCO3* gene is the major gene studied for its role in lipid metabolism (association with higher triglyceride levels), *MTP* gene was studied for its role in regulating synthesis, storage and export of hepatic triglyceride content. A loci on the long arm of chromosome 11 (*11q23*) harbors genes coding for apolipoproteins, including apolipoprotein *A1 (APOAJ), A4 (APOA4* and *APOC3*[23]). Two polymorphisms T455C (rs2854117) and C482T (rs2854116) in the *APOC3* gene either singly or in combination had 30% higher levels of fasting plasma APOC3 and triglyceride levels as compared to the wild type[24]. Subsequent studies failed to replicate these associations[25,26], including our own study[27]. However we found that the SNPs were associated with higher triglyceride levels. *MTP* gene (microsomal triglyceride

Table 2 Functional role of major genes associated with non-alcoholic fatty liver disease identified by genome/exome wide association studies

Gene	Protein	Cellular location[1]	Function[1]	Chromosome location[2]	No. of exons and size[2]	Pathway/biologic function[1]	Tissues expressed[1]
PNPLA3	Patatin-like phospholipase domain-containing protein 3	Lipid droplets	Triacyl glycerol lipase and acylglycerol O-cyltransferase activities	Chromosome 22: 43,923,739-43,964,488 (forward strand)	9 (2805 bp)	Triacyl glycerol degradation and in glycerol-lipid metabolism	Liver, gall bladder, kidney, exocrine pancreas, seminal vesicles, intestine and salivary glands
TM6SF2	Transmembrane 6 superfamily member 2	ER and the ER-golgi intermediate compartment	Regulation of fat in liver influencing triglyceride secretion and lipid droplet content	Chromosome 19: 19,264,364-19,273,391 (reverse strand)	10 (1505 bp)	Promotes very low density lipoprotein export	Liver and intestine
SAMM50	Sorting and assembly machinery component 50 homolog	Outer mitochondrial membrane	Assembly of beta-barrel proteins	Chromosome 22: 43,955,421-44,010,531 (forward strand)	15 (1717)	Transport to the Golgi and subsequent modification and mitochondrial protein import	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
PARVB	Parvin, beta	Cytoplasm	Involved in the reorganization of the actin cytoskeleton and formation of lamellipodia	Chromosome 22: 44,024,277-44,172,949 (forward strand)	13 (5429 bp)	ERK signaling and focal adhesion	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
NCAN	Neurocan	Extracellular, Golgi lumen	Modulates neuronal adhesion and neurite growth during development	Chromosome 19: 19,211,973-19,252,233 (forward strand)	15 (6387 bp)	Developmental	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
PPP1R3B	Protein phosphatase 1, regulatory subunit 3B	Glycogen granule	Acts as glycogen targeting subunit for phosphatase PP1	Chromosome 8: 9,136,255-9,151,574 (reverse strand)	2 (5548 bp)	Regulating glycogen synthesis	Liver, skeletal muscle
GCKR	GCKR	Cytoplasm, nucleus	Inhibits glucokinase by forming an inactive complex with this enzyme. The affinity of GCKR for GK is modulated by fructose metabolites	Chromosome 2: 27,496,842-27,523,684 (forward strand)	19 (2186 bp)	Carbohydrate metabolism	Liver, pancreas, colon and other tissues
LYPLAL1	Lysophospholipase-like 1	Cytoplasm	Depalmitoylating activity	Chromosome 1: 219,173,844-219,212,865 (forward strand)	5 (1898 bp)	Negative regulation of golgi to plasma membrane protein transport	Liver, muscle, skeletal, lung adipocyte, colon and other tissues

[1]Data extracted from http://www.genecards.org/; [2]Data extracted from ENSEBL http://asia.ensembl.org/index.html; ER: Endoplasmic reticulum; PP1: Protein phosphatase 1; GCKR: Glucokinase (Hexokinase 4) regulator; GK: Glucokinase; ERK: Extracellular signal-regulated kinase.

transfer protein), located at 4q24[28] is critical for the synthesis and secretion of VLDL (very low density lipoprotein) in the liver. A meta-analysis[29] of most studied polymorphism -493G > T (rs1800591 G > T) in the *MTP* gene suggested that the SNP was significantly associated with higher risk of NAFLD.

Genes influencing inflammation and immune responses are known to modify susceptibility to NAFLD. Cytokines not only play an active role in the development of disease, but also in the progression by regulating the inflammatory process[30]. Studies identified a positive correlation between increasing degree of liver fibrosis and levels of TNF-α[31-33] including pediatric NAFLD[34]. Further, polymorphism studies associated a promoter SNP (-238G > A) in *TNF-α* gene with susceptibility to NAFLD in Chinese

population[35]. transforming growth factor-beta (TGF-β), known to regulate cell death and lipid metabolism[36], has been shown to be up-regulated and is considered an early event in steatohepatitis that is progressive.

Expression of interleukin-6 (IL-6), a major pro-inflammatory cytokine was shown to be increased in animal models of NAFLD, while in mice, sustained selective up-regulation in the liver resulted in systemic insulin resistance[37]. This was subsequently confirmed in humans[38]. Further, a positive correlation was observed between the expression levels and degree of inflammation and stage of fibrosis. A study identified that -174G/C in the *IL-6* gene was involved in inflammation and insulin resistance and associated with NASH[39], chronic liver disease and Hepatocellular carcinoma[40].

Interleukin-10 (IL-10), an anti-inflammatory cytokine coded by *IL-10* gene[41] has a role in regulating inflammation and its anti-inflammatory properties are well known[42]. T cell, monocyte and macrophage mediated functions are inhibited by IL-10. Different types of the cells in liver including stellate cells, hepatocytes and kupffer cells have shown the presence of IL-10. Few studies that explored the role of the gene, identified the protective role of endogenous role of IL-10 against hepatic steatosis, however they suggested that it does not improve hepatic or whole body insulin sensitivity during high-fat feeding[43]. Furthermore, in an animal model of diet-induced fatty liver disease, inhibition of IL-10 promoted increased expression of inflammatory cytokines, worsened insulin signaling and activated gluconeogenic and lipidogenic pathways[44].

Hepatic insulin resistance is associated with NAFLD and is one of the contributory factors in the pathogenesis of metabolic syndrome. Genetic screening of insulin signaling cascade identified a substitution (Glycine-Arginine) at codon 972 of the insulin receptor substrate-1 gene with a prevalence of approximately 9% in Caucasians that was associated with reduced insulin sensitivity. Furthermore, obese individuals heterozygous for this mutation have 50% reduced insulin sensitivity as compared to wild type obese subjects[45]. This variant is known to affect insulin receptor activity predisposing to liver damage and decreased hepatic insulin signaling in patients with NAFLD. It is suggested that insulin signaling might play a causal role in the progression of liver damage in NAFLD[46].

NAFLD pathogenesis is a complex mechanism with involvement of free fatty acid (FFA) oxidation[47,48] and genes encoding proteins that are involved in the oxidation process of FFAs influence the oxidation load in individuals with obesity, insulin resistance and metabolic syndrome[22]. Genes harboring polymorphisms involved in generation and degradation of reactive oxygen species play a crucial role that could be due to excessive oxidation of FFA leading to oxidative stress casing apoptosis and liver injury[49]. Namikawa *et al*[50] reported that the TT genotype in the *MnSOD* gene, the main ROS scavenger in mitochondria, leads to decreased efficiency

in the transport of MnSOD to the mitochondria and therefore confers susceptibility for NAFLD. Apart from the *MnSOD* gene, substantial evidence is now available on the role of polymorphisms in genes namely *GSTM1, GSTT1* and *GSTP1* genes that are involved in the generation or degradation of ROS. These genes are known to be involved in the progression to cirrhosis[51].

NUTRITIONAL RECOMMENDATIONS

Currently in clinical practice, a combination of vitamin D, vitamin E and omega-3 fatty acids have shown promise in the treatment of NAFLD and seem to be beneficial in patients with NAFLD. Studies further suggests that apart from nutritional counseling that includes a multidisciplinary team (dietician, psychologist, and physical activity supervisor) aerobic exercises, gradual weight loss, management of NAFLD associated conditions namely diabetes, obesity and metabolic syndrome, nutritional recommendations namely use of 400-800 IU/d vitamin E, 1000 IU/d vitamin D, 1 g/d omega-3 fatty acids, and olive oil containing omega-9 fatty acids seem to benefit in reducing the severity of NAFLD. Also, restricting calorie intake to less than 30 kcal/kg per day and including a balanced diet with low levels of saturated and trans fats and simple sugars, avoiding soft drinks with high fructose corn syrup, fast food (trans fats, and reduce red and processed meats), and genetically modified crops seem to be beneficial[52].

MANAGEMENT OF NAFLD

Lifestyle modification usually by way of weight reduction through diet and exercises is currently the only proven strategy for managing NAFLD. As obesity is a strong risk and influencing factor for NAFLD, weight loss (≥ 8% of body weight) is effective and is also the first line of therapy. A low caloric diet with reduction in the intake of total fat, saturated fatty acids, trans fatty acids and fructose, increase in physical activity and abstaining from smoking is advantageous and the patients are encouraged to follow these. Antioxidants, anti-inflammatory, insulin sensitizers, lipid lowering agents apart from wide range of drugs and supplements have been evaluated in various studies, both animal and human, however none of these have efficacy on long term use[53,54].

GENETIC AND NUTRIENT INTERACTIONS

It is almost imperative that all living organisms either simple or complex multicellular, regulate their metabolism vis a vis nutrient availability. Thus, interactions between nutrition and gene are widespread and an ancient feature across species. However, this aspect has not been explored and it is only recently that research started to uncover the mechanism. In view of the rapid advances made in sequencing human genome enormous amount of genetic data is being generated, particularly with
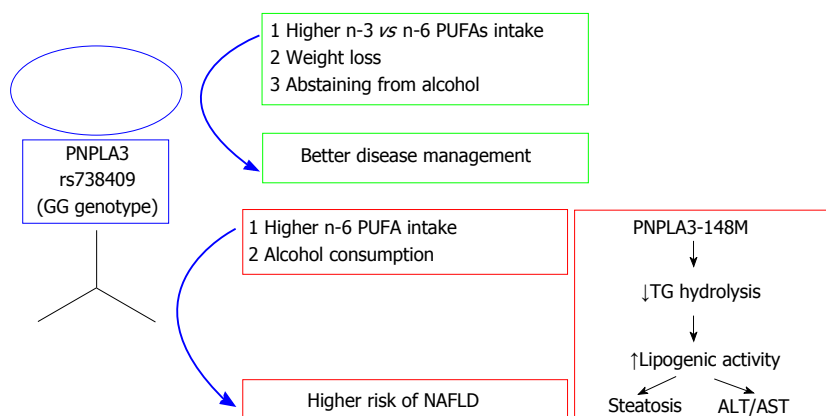
Figure 2 Genotype (rs738409) in patatin-like phospholipase domain containing 3 gene and its interactions. PNPLA3: Patatin-like phospholipase domain containing 3; PUFA: Polyunsaturated fatty acid; NAFLD: Non-alcoholic fatty liver disease; ALT/AST: Alanine aminotransferase/aspartate aminotransferase.

respect to common multigenic, multifactorial conditions including obesity, diabetes, NAFLD, *etc*. It is becoming more and more obvious that an individual's susceptibility to lifestyle disease represents a complex interaction between genetics and environmental interactions. Food and nutrient intake are the important environmental factors and their interactions with genes play a key role in the pathogenesis and progression of polygenic diseases. Therefore, research should be focused on identifying such interactions of genes with nutrients and identifying susceptible genotypes to particular nutrients. This will help us optimize nutrient/diet intake (personalized nutrition) to reduce disease risk[55]. However, there are challenges in analyzing these interactions in the form of genetic heterogeneity and complex nature of human genome, complexity of environmental factors including diet, *etc*[56].

NAFLD is considered to be the hepatic manifestation of the metabolic syndrome[57]. The "thrifty genotype" a possible explanation for the steep increase in obesity and diabetes, where periods of famine in the history of modern humans has exerted natural selection in favor of selecting genes favorable for fat storage and this is likely mediated through fertility and not viability selection[58].

Hepatic lipase gene (*HL*) is a lipolytic enzyme that regulates triglyceride levels. Insulin is known to up-regulate the activity of HL through the insulin-responsive elements in the promoter region. It is suggested that higher intake of total and saturated fat is associated with higher activity of *HL* gene. A study reported that this activity is influenced by the -514 C > T polymorphism in the *HL* gene, with significantly stronger associations noted between total dietary fat intake and HL activity in individuals with CT and TT genotypes as compared to the wild type (CC)[59]. Another study[60] that explored epidemiologic genotype-nutrient interactions in obesity, where a total of 42 SNPs in 26 candidate genes were genotyped identified an interaction between -514 C > T in *HL* gene and fiber intake. Further they also suggested that the -681 C > G polymorphism in *PPARG3* gene might interact with the percentage of energy derived

from fat in the diet for the development of obesity. However, this was a case-only study with only adult obese women as part of the analysis. A study[61] that examined the interactions between the -514 C > T in *HL* gene, dietary fat and HDL-related measures in 1020 men and 1110 women from the farmingham study reported that individuals with the "TT" genotype may have an impaired adaptation to higher animal fat diets. Furthermore, they suggested that dietary fat intake modifies the effect of the polymorphism in *HL* gene on HDL-C concentrations and subclasses, where the T allele was significantly associated with greater HDL-C concentrations only in subjects consuming < 30% of energy from fat and the reverse is true when total fat intake was ⩾ 30% of energy.

It is well established that apolipoprotein A5 (*APOA5*) gene is an important determinant of plasma triglyceride levels. Further it is a component of several lipoprotein fractions including high density lipoprotein (HDL), VLDL[62]. A study[63], investigated the interaction between variants in *APOA5* gene and dietary fat in determining plasma fasting triglycerides, remnant-like particle concentrations and lipoprotein particle size in 1001 men and 1147 women from farmingham heart study reported significant gene-diet interactions between the -1131T > C polymorphism in *APOA5* gene and polyunsaturated fatty acid (PUFA) intake were found that determined fasting TGs, RLP concentrations and particle size. However, these interactions were not found for the other polymorphism (56C > G). Further they noted that the -1131C allele was associated with higher fasting TGs and RLP concentrations in only individuals who consumed a high-PUFA diet with > 6% of total energy. The study concluded that individuals who are carriers of -1131C polymorphism in *APOA5* gene and take higher n-6 but not n-3 PUFA, have increased fasting TGs, RLP concentrations, and VLDL size and decreased LDL size, suggesting a more atherogenic lipid profile in these individuals because of the n-6 PUFA-rich diet.

A study[64] that recruited 8 subjects with homozygous genotype for the rs738409G allele in *PNPLA3* gene and

10 with C allele, explored the influence of the variant on the ability to lose weight thereby reducing liver fat or change insulin sensitivity. The study identified that the fasting serum insulin and C-peptide concentrations were significantly lower in rs738409G as compared to rs738409C group. Although weight loss was not significantly different between the groups (approximately 3.1 kg), liver fat decreased by 45% in rs738409G as compared to 18% in the rs738409C group, suggesting weight loss is more effective in decreasing liver fat in rs738409G carriers. Another study[65] that explored the influence of PNPLA3 (rs738409) genotype on hepatic fat and modulation by dietary factors such as PUFAs identified that the ratio of n-6 to n-3 PUFs interacted with the GG genotype to promote hepatic steatosis (Figure 2).

FUTURE TRENDS

In future, testing for variants that pre-dispose to NAFLD along with their nutrient interactions would help identify the type of nutrition to be taken based on individual's genetic makeup thereby minimizing the risk of fatty infiltration.

CONCLUSION

Among all the loci identified thus far, there is a compelling evidence of the association of variants in PNPLA3 with NAFLD and functional role of TM6SF2 in the regulation of liver fat metabolism and hepatic lipid droplet content. It may be prudent to genotype these well characterized variants (PNPLA3) as part of the diagnostic workup for NAFLD, to assess the risk of an individual. Further genotyping in asymptomatic individuals will help in making lifestyle based recommendations, including nutrition to minimize the risk of future disease. As the genetic susceptibility risk cannot be changed, it is important to identify the risk at an early age and manage/lower the other modifiable risks/modifiable triggers to efficiently manage the disease without progressing to subsequent pathologies. Finally, the genetic information along with personalized environment exposures will help in stratifying risk of NAFLD in an individual.

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