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GENETICS (GVZ DEDOUSSIS, SECTION EDITOR)

The Genetics of Nonalcoholic Fatty Liver Disease: Role of Diet as a Modifying Factor

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Abstract Nonalcoholic fatty liver disease (NAFLD) is defined as the hepatic manifestation of the metabolic syndrome, and thus it is mainly linked to excess body weight and systematic insulin resistance. Moreover, recently published data are indicative of the contribution of environmental factors and susceptible genetic background in NAFLD onset and progression. PNPLA3 rs738409 is the main variant studied for NAFLD so far, and diet is recognized as the drastic environmental exposure. Research on the field is preliminary but promising. This review meets the need of a summary of all available data; it describes the role of specific genes and dietary constituents, as well as the nutrigenomic and nutrigenetic effects on NAFLD. Studies in the area hold promise for future personalized diet interventions on the reduction of NAFLD and related health problems incidence.

Keywords NAFLD · NASH · Diet · Nutrients · Gene · Interaction · Modifying · Factor · Nonalcoholic · Fatty · Liver · Disease · Steatohepatitis · SNP · Polymorphism · PNPLA3 · Nutrigenomics · Nutrigenetics

Introduction

During the past decade, the pathogenesis and background of nonalcoholic fatty liver disease (NAFLD) has attracted broad interest from scientists. NAFLD is now known as the hepatic manifestation of the metabolic syndrome, in the absence of excess alcohol intake [1]. Since metabolic factors, such as obesity and diabetes mellitus II (T2DM), have rapidly increased, NAFLD tends to become an epidemic. Numbers so far report that the prevalence of the disease is 20-30 % in the general Western population and 10-20 % in the Eastern [1].

NAFLD constitutes an asymptomatic disease, which in some cases progresses to severe liver damages, such as nonalcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis. It is established when triglycerides (TAG) are excessively accumulated in the liver, leading to oxidative stress, immune cell response, and ultimately to liver morbidity. The multiple-hit model has now predominated as the NAFLD pathogenesis theory [2].

Apart from metabolic parameters, genetic and environmental factors also are considered independent contributors to the onset and progression of NAFLD. Moreover, the fact that the disease is present in 25 % of the normal-weighted European individuals supports this claim [3].

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Role of Genetics in NAFLD

The past decade has been proved very revealing about the genetic background of NAFLD. The first early evidence of the role that genes play in the development of this disease was reported in 2000, when Struben and colleagues noticed that NASH and cryptogenic cirrhosis coexisted in the vast majority of the families studied [4]. Since then, family, twin, and interethnic studies have confirmed the genetic risk for NAFLD [5, 6]. Apart from the different living conditions and differences in individuals' metabolic profile (obesity/fat distribution, insulin resistance/T2DM), which partly explain the various disease prevalence, different genetic backgrounds also have been reported.

Due to its complicated pathogenesis, candidate-gene studies have only poorly revealed NAFLD-associated SNPs [7]. Since the human genome sequence publication, genetic association studies have increased. Thereafter, genome-wide

association studies (GWAS) shed light on novel genetic loci, never previously studied for NAFLD.

PNPLA3

At the time of writing, there is one gene that has mostly attracted the scientific interest: PNPLA3, a gene located on 22q13.31. It encodes a 481 amino acid protein named patatin-like phospholipase domain-containing 3, also known as adiponutrin (ADPN). ADPN is a triacylglycerol lipase that mediates triacylglycerol hydrolysis in hepatocytes and adipocytes; it is mostly bound on cell membranes and lipid droplets. PNPLA3 expression increases in the fed state, whereas in the fasted state remains low [8]. It also has been suggested that in the postprandial phase, insulin and fatty acid composition of the food regulate PNPLA3 expression.

A strong association of a PNPLA3 polymorphism (rs734809) with hepatic fat accumulation, nonaccompanied by features of metabolic syndrome, was first reported in a GWAS study by Romeo et al. in 2008, and it was then replicated by other GWAS and candidate-gene studies in both children and adults [7, 9]. The rs738409 polymorphism is caused by a cytosine to guanine change, leading to an isoleucine substitution with methionine at codon 148 (I148M). It also has been associated with liver enzymes levels [9, 10]. Romeo et al. also detected a PNPLA3 variant (S453I), more frequent in the African-American group, which was associated with less hepatic TG accumulation. Later, this PNPLA3 variation was associated with liver steatosis and fibrosis [11–13].

It is now certain that PNPLA3 plays a modifying role in NAFLD pathogenesis and progression. However, little is known about the physiological role of the altered protein. There are three major suggestions about the risk allele role, involving: a) the overexpression and accumulation of free fatty acids and TAG in the hepatocytes, b) an impaired TAG hydrolysis and VLDL synthesis, or c) a relative depletion of TAG long-chain polyunsaturated fatty acids [14]. To conclude, the pathogenesis and mainly the progression of NAFLD need more research in order to be clarified.

Studies have revealed various other potential genetic modifiers. Below are discussed genes that have been replicated by independent cohorts.

GCKR

Mutations in the glucokinase regulator gene (GCKR) alter its ability to control glucose metabolism [15]. Two large GWAS first identified a polymorphism on GCKR gene, the rs780094, which was associated with NAFLD [16, 17]. It is now believed that the effect of this SNP is attributed to its proxy SNP rs1260326; the latter has been linked to impaired GCKR ability, continuous glucose uptake by the liver and therefore perpetual hepatic glycolysis, acetyl-CoA synthesis, and hepatic lipogenesis [18]. GCKR rs780094 was later associated with significant liver fibrosis and high serum TAG levels [19]. A joint effect on NAFLD susceptibility of GCKR SNPs and PNPLA3 SNP also has been reported in both children and adult populations [20–23]. Recently, an interesting interaction between the insulin-increasing rs780094 allele and whole grain intake has been introduced [24], suggesting a potential involvement in NAFLD.

AGTR1

Angiotensin II is believed to cause liver injury by promoting fibrogenesis [25, 26]. Studies in rat models have shown that AGTR1 blockers can control the progress of NASH and fibrosis [27, 28]. In a case-control study, Yoneda et al. identified an AGTR1 gene polymorphism (rs3772622) and four more SNPs in LD with it that were strongly associated with NAFLD and fibrosis [29]. Four years later, Zain et al. stated a gene-gene interaction between AGTR1 and PNPLA3 genes and a protective effect of AGTR1 SNPs against NAFLD in an Indian population, which is in contrast with Yolenda's results [30].

MTTP

Microsomal triglyceride transfer protein (MTTP) regulates TG circulation and lipoprotein assembly and secretion in the liver. Studies about the MTTP polymorphism −493 G/T and NAFLD risk have failed to agree [31]. However, there is some evidence that the wild type (GG) is more frequent in NASH patients and that G allele carriers are at risk for more severe steatosis [32, 33].

PEMT

Phosphatidyl ethanolamine methyltransferase (PEMT) enzyme acts in the liver by catalysing the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC), required for normal hepatic VLDL secretion. In the past, two studies reported that carriers of the Val175Met allele of PEMT gene had an increased risk to develop NAFLD [34, 35]. On the other hand, Romeo et al. failed to show any association [36].

APOC3

APOC3 interferes with the uptake of lipoproteins in the liver and inhibits lipoprotein lipase activity. So far, one study has reported the association of two APOC3 polymorphisms (rs2854116, rs2854117) with NAFLD risk [37]. Four more scientific groups looked into those SNPs, but none of them replicated the association [38–41].

SOD2

Mutations in the SOD2 gene cause a reduction in MnSOD mitochondrial transport and can lead to cellular damage. The SOD2 C47T SNP (rs4880) has been found to be more common among Japanese NASH patients [32]. However, a recent, European, case-control and family study reported that the SOD2 SNP is associated with degree of steatosis and advanced fibrosis, but not with NASH [42].

KLF6

KLF6 is a transcription factor, functioning as a regulator of several fibrogenesis-related genes. KLF6 rs3750861 is believed to delay histological progression of NAFLD [43]. Furthermore, recent data showed that carriers of the effect allele had milder insulin resistance than the wild type homozygous [44].

Exome Wide

Exome-wide sequencing is a new, promising approach of identifying potentially causative missense variants. It was recently applied on 2,736 participants of the Dallas Heart Study, and, apart from two PNPLA3 variants, there was only one polymorphism in TM6SF2 gene (Glu167Lys, rs58542926) that exceeded the exome-wide level of significance. The biological role of TM6SF2 remains unknown [45]. Whole-exome sequencing has been performed on ten obese Caucasian patients; one was found to be homozygous for rare damaging PNPLA3 mutations [46].

Role of Diet in NAFLD

The development of NAFLD is associated with long-term excessive food consumption and dietary composition in specific food groups, macronutrients, and micronutrients. Several studies have assessed dietary habits of patients, supporting their adherence to a specific pattern and examining potential benefits from dietary modification.

Dietary Habits

A consecutively increased energy intake leads to positive energy balance and consequently in obesity and its metabolic disorders. This state clearly correlates with NAFLD with likelihood of disease progression increasing in parallel with obesity degree [47]. A systematic review that examined the efficacy of trials that caused 4-14 % weight loss via reducing energy intake and/or increasing physical activity concluded that lifestyle modification reduces hepatic lipid accumulation and improves insulin sensitivity [48]. Although results are promising, adherence to a long-term weight control program is difficult for many obese patients.

Fast-food consumption is a modern Western trend, correlating with increased total daily energy intake, poor nutrient intake, and higher soft drink consumption [49]. According to CARDIA study, frequent fast food consumption at baseline and follow-up caused, after 15 years, an extra weight gain of 4.5 kg and a twofold greater increase in insulin resistance [50]. Moreover, a 1-month, fast-food–based hyperalimentation led to a pathological increase in ALT levels in most subjects and an increase in hepatic lipid accumulation and insulin resistance, suggesting a possible effect on NAFLD [51]. These findings are further supported by a trial in which mice fed a fast-food diet developed obesity, insulin resistance, and NASH with progressive fibrosis [52].

Furthermore, consumption of regular soft drinks, sweetened with fructose in the form of sucrose or with high fructose corn syrup (HFCS), is increasing worldwide [53]. Fructose intake is precisely two- to threefold higher in NAFLD patients [54]. It increases de novo lipogenesis, alters carbohydrate homeostasis, and mediates inflammation [55], thus provoking NAFLD. Moreover, excessive soft drink consumption leads to dyslipidemia and visceral adiposity and decreases insulin sensitivity [56]. However, the results of a systematic review that examined interventions in which fructose was exchanged isocalorically with other carbohydrates or provided excess energy, were conflicting. Intrahepatocellular lipids and ALT levels increased only on hypercaloric trials [57]. It remains unknown whether this was due to excessive energy intake or increased fructose consumption.

Macronutrients and Micronutrients

Fat

Fat intake is higher in NAFLD patients acting as an independent risk factor of the disease [58]. Moreover, NASH patients compared with those with simple steatosis have a greater intake, suggesting its potential role in NASH development [59]. However, fat quality may be more linked to NAFLD than its amount.

Saturated fatty acids (SFA) intake is higher in NASH patients than in controls [60]. A considerable portion of hepatic TAG is derived from diet [61] and increased hepatic saturated fatty acids correlate with greater liver injury [62], possibly provoking progression from hepatic steatosis to NASH [63]. SFA intake also correlates with increased risk of cardiovascular disease, further supporting an adverse effect on NAFLD [64].

Concerning monounsaturated fatty acids (MUFA), there are conflicting reports of their intake from NAFLD patients. It has been observed that the dietary intake is below the

recommended level [65, 66], whereas some studies have failed to point a difference [60, 67]. MUFA intake correlates with lower risk of cardiovascular disease via improving lipid profile and blood pressure and limiting LDL oxidation [68, 69]. Moreover, weight maintenance with MUFA-rich diet improves insulin resistance [70] and an animal investigation showed that olive oil consumption decreases hepatic fat accumulation [71]. These findings suggest a potential role in NAFLD, but more research is needed.

A diet poorer in polyunsaturated fatty acids (PUFA), higher intake of n-6 fatty acids, and higher n-6/n-3 ratio characterize NASH patients [60, 66]. Similarly, NAFLD patients compared with controls consume more meat, which contains n-6 fatty acids, and tend to consume less fish rich in n-3 fatty acids [67]. Considering the anti-inflammatory effect of n-3 fatty acids, the proinflammatory and prothrombotic action of n-6 fatty acids, and the inflammation that characterizes advanced NAFLD, this imbalance may cause disease progression [72]. Animal data indicate that n-3 fatty acids can reduce hepatic steatosis, inflammation and oxidative stress, and improve insulin sensitivity [73–75]. According to a systematic review, n-3 fatty acid supplementation (0.8-13.7 g/day) beneficially changes liver fat without adverse effects, suggesting a role in decreasing hepatic steatosis [76]. More research is required to assess their efficacy and safety as treatment and to determine their ideal dosage.

Transfatty acids intake has adverse effect on cardiovascular disease and insulin resistance, conditions associated with NAFLD [77]. Transfat-fed mice compared with lard-fed ones developed greater hepatic steatosis and hepatocellular injury [78] and compared with those fed PUFA and SFA diets have greater fat accumulation, which correlates with NASH inflammation [79]. Therefore, transfatty acids intake may have an important role in NAFLD.

Protein

There are conflicting results regarding protein intake. A lower but otherwise adequate intake characterizes NAFLD patients [66]. In a recent study, a hypocaloric, high-protein diet improved lipid profile, glucose metabolism, and liver enzymes even without weight loss [80]. It is suggested, although that type of dietary protein is important considering that soy protein reduces hepatic lipotoxicity and increases insulin sensitivity while casein has the opposite effect [81]. Replacement of casein by soy protein can effectively lower hepatic lipid accumulation [82].

Carbohydrates

NAFLD patients are characterized by higher carbohydrate consumption [58]. Among obese patients, carbohydrate intake at the highest limit of the dietary allowance range correlated with increased risk of liver fibrosis [83]. Thus, low carbohydrate diets have been used as intervention therapy in NAFLD. Despite their efficacy in favoring weight loss and reducing intrahepatic TAG, long-term adherence induced adverse effects on metabolism of mice, including NAFLD and insulin resistance [84]. Carbohydrate type is probably more important than the consumed quantity itself.

Vitamins E and D

NAFLD patients are characterized by decreased vitamin E intake [60] and plasma concentration compared to healthy individuals [85]. Its potential therapeutic role as antioxidant has been investigated in NAFLD. TONIC and PIVENS trials administered vitamin E at a dosage of 800 IU/day [86, 87]. In the PIVENS study the supplementation group had higher rate of improvement in NASH and lobular inflammation, and in the TONIC study it had greater NASH resolution compared with placebo. However, there is evidence that its supplementation increases risk of hemorrhagic stroke [88], prostate cancer [89], and possibly overall mortality [90, 91].

Vitamin D serum levels are low in NAFLD patients and correlate with histological severity of the disease independent of metabolic characteristics [92, 93]. According to a systematic review, vitamin D deficiency may negatively influence glycaemia [94], and as NHANES III showed, it is associated with increased risk of prevalent cardiovascular disease [95]. Considering the adverse effect of vitamin D deficiency, further research is needed to explore a potential benefit from its supplementation in NAFLD patients.

Choline

Choline is an essential nutrient, abundant in diet [96]. Its deficiency leads to hepatic lipid accumulation and organ dysfunction in most subjects, conditions reversible after choline administration [97, 98]. Its effect on the liver ranges from simple steatosis to hepatocellular carcinoma [96]. However, dietary requirement varies among individuals due to genetic variation, estrogen status, and microbiome composition.

Other Dietary Constituents

Recent literature suggests that whole-grain products [60, 99–101], cholesterol [60, 102–104], coffee caffeine [105, 106], alcohol [107], taurine [108], anthocyanins [109], and catechins of green tea [110] also may have a role in NAFLD management. More research is needed to elucidate their effect.

Gene-Diet Interactions in NAFLD/NASH

Few studies have looked into diet as a modifier of NAFLD-related genes expression in humans, whereas most published studies are applied on mouse models. Figure 1 depicts the most studied gene–diet interactions concerning NAFLD/NASH in both humans and animal models.

So far, one study has examined the interaction of dietary and lifestyle habits with oxidative-stress–associated gene expression in 583 NAFLD adult patients and controls [111••]. An increased risk for NAFLD was found when risk allele carriers of glutathione S-transferase Mu 1 (GSTM1), glutathione S-transferase theta 1 (GSTT1), CYP1A1 and CYP2E1 of the cytochrome P450 superfamily, and sulfotransferase 1A1 (SULT1A1) genes reported a consumption of more than two fruits per day. In addition, risk allele carriers of GSTM1, GSTT1, CYP1A1, and SULTI1A1 had an increased risk for NAFLD when the consumption of grilled meat or fish was more than once per week. On the other hand, it was observed that moderate alcohol consumption in carriers of the CYP2E1*5B allele was protective. A gene-gene interaction also was demonstrated between the $CYP2E1_{PstI}$ and GSTM1 genes, for those carrying the minor alleles (odds ratio [OR]= 0.21). However, the study was underpowered, and a larger sample size is necessary to replicate these results.

Recent works have investigated the relationship between a high-fat diet (HFD) and diet-reactive hepatic gene expression in mice. Comparing a HFD with a low-fat diet in SM/J and LG/J inbred mouse strains, Partridge et al. found 259 immune response and oxidative stress-related genes whose hepatic expression was changed due to the HFD effect [112]. Waller-Evans et al. studied the HFD effect versus a carbohydrate control diet in C57BL/6J and BALB/c mice strains for 23 weeks. HFD fed C57BL/6J was found to be prone to insulin resistance and NAFLD, whereas HF fed BALB/c were relatively resistant, due to lower fatty acid uptake. PPARγ, proteasome, and the ubiquitin-mediated proteolysis pathway were conversely expressed between the two strains [113]. In a recently published paper, a western maternal diet when combined with a western offspring diet resulted in hepatomegaly, elevated hepatic ALT, AST, TG, and TC levels, and increased expression of TNFa, CD11, MCP1, and TGFB compared with a low-fat diet [114]. Epigenetic changes also were detected. Moreover, when HFD diet is combined with alcohol consumption (concentration up to 5 %), proinflammatory and profibrotic genes are overexpressed compared with the HFD

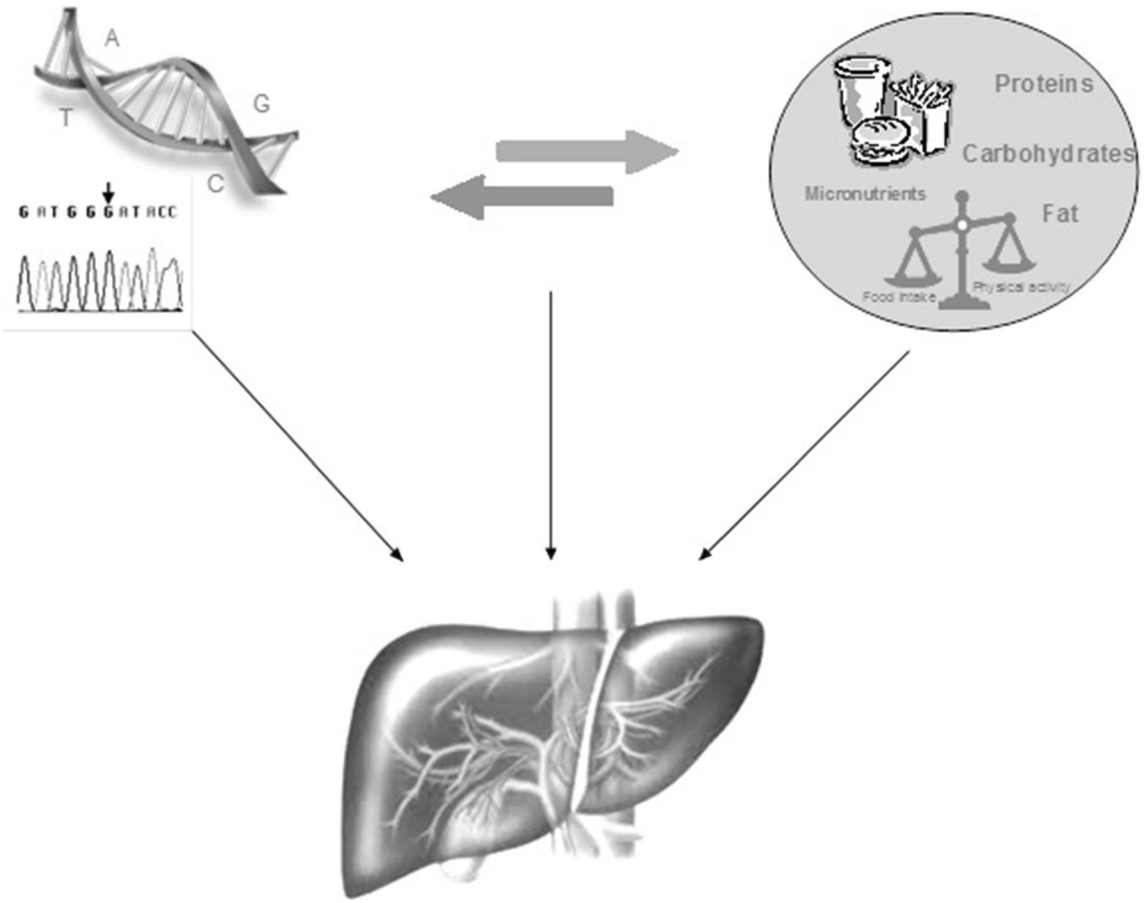

Fig. 1 Gene-diet interactions in NAFLD/NASH

alone in BALB/c mice [115]. This is an interesting result, because this strain is believed to be relatively resistant to fatty liver. The type of dietary fat consumed also has been examined for possible interactions with gene expression in NAFLD development. In a recent human study, increased n-6/n-3 PUFA ratio interacted with PNPLA3 rs738409 in the GG homozygotes influencing ALT levels and hepatic fat accumulation [116••]. It seems that n-6 fatty acids lead to oversynthesis of proinflammatory species, which trigger inflammation leading to NASH. Moreover, it has been observed that severe hepatic steatosis was more probable among GG homozygotes of PNPLA3 rs738409 supplemented with PUFAs compared to heterozygotes, suggesting that G allele is linked to lower response to PUFA supplementation [117••]. N-3 fatty acids act as regulators of hepatic gene expression by mainly targeting the transcription factors PPARa, SREBP-1c, and ChREBP and downregulating inflammatory genes [118]. Human biopsies of NAFLD patients indicated that those with n-3 fatty acids depletion were characterized by decreased expression of PPARa and increased expression of SREBP-1c, a condition favoring lipogenesis over fatty acid oxidation [119]. Supplementing with n-3 fatty acids has been observed to increase PPARa expression and decrease SREBP-1c and ChREBP expression [74, 120] as well as to decrease TNFa levels [73, 121•].

The effect of carbohydrate feeding on NAFLD is possibly mediated by the activation of SREBP-1c, a transcription factor that enhances the expression of enzymes associated with fatty acid synthesis and activates PNPLA3 [8]. An animal study demonstrated a 90-fold PNPLA3 upregulation when carbohydrates were introduced after fasting [8]. In humans, a recent nutrigenetic analysis in 153 Hispanic children, aged 8-18 years, indicated a positive association of total sugar and carbohydrate intake with hepatic fat deposition only in the GG homozygotes for the PNPLA3 rs738409 [122•]. In line with this, another study observed an interaction in steatosis severity between moderate or excessive consumption of soft drinks, which mainly contain fructose, and the PNPLA3 rs738409 allele [123••]. The strongest association concerned those who consumed soft drinks at least once weekly. A recent study suggested that PNPLA3 also might be regulated in human liver by glucose via ChREBP [124]. Moreover, it has been observed that fructose rather than glucose administration decreases hepatic fatty oxidation and increases proinflammatory transcription NF-KB activity, thus causing hypertriglyceridemia and hepatic steatosis [125]. The mechanism for fructose induced hepatic steatosis has been shown to be dependent on ketohexokinase C enzyme [126]. Application of a low carbohydrate diet (LCD) on 18 NAFLD adult patients for 6 days showed a stronger liver fat reduction in the PNPLA3 I148M GG group, despite the same rates of weight loss [127]. Nobili et al. reported a weaker predisposing effect of this mutation on steatosis severity for those with poor vegetable intake [123••]. These results demonstrate that GG individuals benefit more than C allele carriers from a LCD.

Concerning protein intake, a 35 % protein diet in liver steatosis-induced mice reversed steatosis and reduced hepatic lipid concentrations, independently of fat and carbohydrate intake [128]. This change was more effective than a 20 % reduction in energy intake and it was linked to small increases in TCA cycling, lipid, and branched-chain amino acids catabolism. So far, no studies have tested the nutrigenomic effects of different protein types.

It is now believed that choline, as a methyl-donor, is important in NAFLD pathogenesis, possibly due to the role of PC in VLDL secretion from liver [96]. Moreover, choline is considered to cause epigenetic changes and recent studies explain how these changes cause liver fibrosis in a low-methyl environment [129–132]. Furthermore, Spencer and colleagues have suggested a model that could accurately predict the possibility of an individual to develop NAFLD while being on a choline-deficient diet. This model combines the gut microbiome synthesis and the presence of the PEMT rs12325817 polymorphism [133]. On the other hand, supplementation of a choline and methionine poor diet with vitamin E was associated with reduced expression of fibrosis, inflammation, and apoptosis genes [134]. Vitamin E supplementation alone increased the expression of adiponectin through the regulation of PPARγ [135]. Low adiponectin levels have been associated with NAFLD [136]. The exact choline and vitamin E roles in NAFLD pathogenesis needs to be further investigated.

Polyphenols, biotin, and niacin supplementation have been studied for their effect in gene expression of NAFLD-induced mice and results are promising [137–143]. In addition, a vitamin D-depleted diet has been positively associated with higher inflammation and oxidative stress genes expression in a rat model [144].

Conclusions

The evidence reviewed in this manuscript indicates that we are in the beginning of understanding the NAFLD pathogenesis and progression. More human studies are needed to clarify the role of the novel PNPLA3 gene and other genes involved in the lipid and glucose metabolism, insulin resistance, oxidative stress, immune cells response, inflammation and hepatic fibrogenesis. Furthermore, intervention trials, population, and family studies will provide us with important knowledge on the diet effect and diet-induced gene dysregulation in NAFLD. As the prevalence of NAFLD steadily increases, it is becoming vital that pathways responsible for individual variability and susceptibility to the disease are revealed and personalized therapies for NAFLD patients are designed.

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Compliance with Ethics Guidelines

Conflict of Interest Ioanna-Panagiota Kalafati, Dimitra Borsa, and George V.Z. Dedoussis declare that they have no conflict of interest.

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- •• Of major importance

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