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Proteomic and genomic studies of non-alcoholic fatty liver disease - clues in the pathogenesis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a widely prevalent hepatic disorder that covers wide spectrum of liver pathology. NAFLD is strongly associated with liver inflammation, metabolic hyperlipidaemia and insulin resistance. Frequently, NAFLD has been considered as the hepatic manifestation of metabolic syndrome. The pathophysiology of NAFLD has not been fully elucidated. Some patients can remain in the stage of simple steatosis, which generally is a benign condition; whereas others can develop liver inflammation and progress into non-alcoholic steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. The mechanism behind the progression is still not fully understood. Much ongoing proteomic researches have focused on discovering the unbiased circulating biochemical markers to allow early detection and treatment of NAFLD. Comprehensive genomic studies have also begun to provide new insights into the gene polymorphism to understand patient-disease variations. Therefore, NAFLD is considered a complex and multifactorial disease phenotype resulting from environmental exposures acting on a susceptible polygenic background. This paper reviewed the current status of proteomic and genomic studies that have contributed to the understanding of NAFLD pathogenesis.

For proteomics section, this review highlighted functional proteins that involved in: (1) transportation; (2) metabolic pathway; (3) acute phase reaction; (4) anti-inflammatory; (5) extracellular matrix; and (6) immune system. In the genomic studies, this review will discuss genes which involved in: (1) lipolysis; (2) adipokines; and (3) cytokines production.

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Key words: Non-alcoholic fatty liver disease; Proteomics; Genomics; Metabolic syndrome; Pathophysiology

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a widely prevalent hepatic disorder in Western populations. NAFLD can occur as a spectrum diseases, from simple steatosis, to non-alcoholic steatohepatitis characterised by hepatocellular injury and inflammation, to cirrhosis and hepatocellular carcinoma. This paper reviewed the current status of proteomic and genomic studies that have contributed to the understanding of NAFLD pathogenesis. This review highlighted several functional proteins and genetic polymorphisms; particular those involved in insulin resistance, triglycerides metabolism and hepatic inflammation. It is hoped that this review will offer further insights into the pathophysiology of NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a widely prevalent hepatic disorder, affecting up to 30% of the

general population of Western countries^[1]. The term NAFLD comprises an entire pathological spectrum of diseases, ranging from simple steatosis (SS), non-alcoholic steatohepatitis (NASH), progressive inflammation, fibrosis, cirrhosis to hepatocellular carcinoma (HCC)^[2]. Furthermore, NAFLD has been shown to be closely associated with obesity, insulin resistance, dyslipidemia, type II diabetes mellitus (T2DM) and cardiovascular disease^[2-4]. Therefore, NAFLD has been considered as the hepatic manifestation of metabolic syndrome (MetS).

In the majority of patients, SS is a relatively benign course. However, SS may evolve into NASH, which is a more aggressive liver disease that tends to be progressive and may lead to cirrhosis^[5]. The mechanism behind the progression of SS to NASH is still not fully understood. Currently, liver biopsy remains the gold standard in diagnosis of NAFLD. The histological hallmark of NAFLD is lipid accumulation in the hepatocytes in absence of pathologies such as viral hepatitis or alcohol abuse^[4]. However, liver biopsy is an invasive procedure and the liver is not necessarily uniformly affected in NAFLD^[6,7]. Therefore, non-invasive biomarkers for evaluation of liver disease and fibrosis are urgently needed.

Proteomics is a large-scale analysis of protein changes of different patient populations. The identification of specific proteins, either as novel markers or as over/under expressed markers, may have a huge impact by increasing the availability of biomarkers for early diagnosis and therapy^[8]. On the other hand, genome-wide association (GWA) studies aim to identify the genetic influences on common diseases with common genetic variation of observable traits. The technologies of high-throughput genotyping are able to assay the common single nucleotide polymorphism (SNPs) and relate them to clinical conditions and measurable traits^[9].

The development of NAFLD is a complex multifactorial process involving the perturbation of multiple gene and protein regulations. Initially, Day and James proposed a “two-hit hypothesis” to explain the concept of steatohepatitis: first hit is the production of steatosis and second hit is the source of oxidative stress capable of initiating significant lipid peroxidation^[10]. However, the traditional “two-hit hypothesis” has been modified to “multiple parallel hits hypothesis”. The significant overlaps among insulin resistance, hepatic *de novo* lipogenesis and subsequent hepatocyte injury have been proposed to contribute to the progression from SS to NASH^[11]. Furthermore, numerous candidate gene studies on the effects of genes on the presence of NAFLD and its progression have further supported the “multiple parallel hits hypothesis”^[12]. This review aims to summarise the current proteomic and genetic studies to provide better understanding about the pathogenesis of NAFLD.

HEPATIC FIBROGENESIS DEVELOPMENT

Accumulating laboratory evidence suggests that insulin resistance and excess of free fatty acids can trigger the

oxidative stress in hepatocytes and lead to chronic inflammation and fibrogenesis^[13,14]. Hepatic fibrogenesis is the final consequences of chronic liver disease and constitutes a model of wound-healing process. Regardless of the cause of hepatic damage, hepatic stellate cells (HSCs) are activated within the extracellular matrix (ECM) into myofibroblasts (activated HSCs). These activated HSCs result in the deposition of fibrotic matrix. Hepatic macrophages have been long recognised to be the source of matrix metalloproteinases (MMP) which can degrade the scar tissue in liver^[15]. The development of fibrogenesis is a dynamic process due to an imbalance in ECM deposition and degradation. Hepatic fibrosis is associated with increased ECM, which include collagen, laminin, hyaluron, elastin and fibronectin. As fibrogenesis progresses, the quantity and composition of ECM changes^[16]. Therefore, the protein expression may vary at different stages of fibrogenesis due to endogenous adaptive responses. Targeting the optimum levels of different protein expression may shed a light in both disease prediction and disease mechanism.

PROTEOMIC STUDIES

The advancement of proteomics analysis has provided us with powerful tools for studying NAFLD diagnosis and discovering biomarkers. The first study of serum protein profiles in NAFLD was published by Younossi *et al.*^[17]. The author performed a surface-enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF MS) on 98 obese patients; where 91 patients were diagnosed with NAFLD (12 steatosis alone, 52 steatosis with nonspecific inflammation, 27 NASH) and 7 patients without NAFLD as obese control. The proteomic analysis revealed 12 significant protein peaks. Due to the inherent limitation of low mass accuracy in SELDI-TOF MS, only fibrinogen γ was identified and suggested that it may be related to fibrosis. Later, Bell *et al.*^[18] utilised an ion-intensity based, label-free quantitative proteomics approach (LFQP) and discovered 55 proteins that changed significantly between NAFLD and NASH with advanced fibrosis. Bell *et al.*^[18] further revealed 15 proteins that changed significantly between early NASH and NASH with advanced fibrosis. A 6 proteins diagnostic method (fibrinogen β chain, retinol binding protein 4, serum amyloid P component, lumican, transgelin 2 and CD5 antigen-like) and a 3 proteins diagnostic method (component C7, insulin-like growth factor acid labile subunit and transgelin 2) were developed to diagnose the different stages of NAFLD (AUROC ranging from 0.83 to 0.91). Additionally, alanine aminotransferase (ALT) has been found to be significantly inferior in diagnosis the stages of NAFLD (AUROC = 0.53)^[18]. Several studies have subsequently demonstrated that ALT is a poor NAFLD workup marker and there is no optimal ALT levels to predict advanced fibrosis^[19]. Despite the lack of single protein in differentiating SS and NASH, Bell *et al.*^[18] have provide an insight into the pathogenesis of NAFLD and

NASH. Interestingly, most of the proteins identified in the study were involved in immune system regulation, inflammation, hepatic ECM structure and protein carriers in blood.

Protein carriers

Apolipoproteins, are important structural and functional proteins that transport lipids in blood circulation. As NAFLD is considered the hepatic manifestation of MetS, it is not surprising that apolipoprotein serum levels are altered in patients with chronic liver disease. Previous publications have revealed that the level of ApoA1 decreased with progression of hepatic fibrosis^[20-22]. A recent proteomic study conducted by Choe *et al*^[21] using rate nephelometry and particle-enhanced immunonephelometry in non-diabetic patients has concluded that ApoB/ApoA1 ratio was associated with the prevalence of NAFLD and was independent of obesity and other metabolic components. Therefore, ApoB/ApoA1 ratio can be used as a predictable marker for cardiovascular risk in NAFLD patients.

CD5 antigen-like (CD5L) protein, also known as human Sp alpha, is a soluble protein that is expressed by macrophages present in lymphoid tissues and may have an important role in the regulation of the innate and adaptive immune systems^[23]. A serum proteomic study using 2-dimensional differential in gel electrophoresis (DIGE) was performed on immune depleted sera from 3 groups of patients (pre-cirrhotic NAFLD, cirrhotic NAFLD and cirrhotic NAFLD with co-existing HCC)^[22]. This study has identified a pattern of serum apolipoproteins (ApoA1, Pro-ApoA1 and ApoA4) and CD5L to distinguish between pre-cirrhotic NAFLD and cirrhotic NAFLD^[22]. However, CD5L does not increase incrementally with the degree of fibrosis and still falls short as cirrhosis marker with AUC of 0.719^[22]. Moreover, CD5L is a poor biomarker for HCC. Hence, there could be another trigger factors from the progression of NAFLD, NASH to HCC.

Metabolic pathways

Rodríguez-Suárez *et al*^[24] analysed the protein expression of liver samples with DIGE in combination with MALDI TOF/TOF. Ten of the proteins were further validated by Western blotting to confirm the observed changes of protein expression^[24]. This study has successfully demonstrated that both serum concentration of carbamoyl phosphate synthase 1 (CPS1) and 78 kDa glucose-regulated protein (GRP78) decreased gradually from cholecystectomy controls with normal liver function and histology subjects to steatosis and NASH patients^[24]. Further validation of CPS1 and GRP78 in higher number of patients is still warranted. However, this study has provided a useful insight for NAFLD progression.

GRP78, also known as immunoglobulin binding protein (BiP), is a central regulator of endoplasmic reticulum (ER) function, which is crucial for cells survival. GRP78 has been recognised for its roles in protein folding, mis-

folded protein targeting for degradation and ER Ca^{2+} homeostasis^[25,26]. Laboratory researches further demonstrated that GRP78 can inhibit both insulin-dependent and ER-stress-dependent SREBP-1c proteolytic cleavage that plays an important role in *de novo* lipogenesis^[27]. In addition, compelling laboratory evidences have indicated that mitochondrial abnormalities may be involved in the pathogenesis of NAFLD^[28-30]. CPS1 is a ligase enzyme located in the mitochondria that involved in the production of urea. The down-regulation of CPS1 may lead to an increase of urea, which subsequently becomes uric acid^[31]. This mechanism could potentially explain the observed significant elevated uric acid levels in NAFLD patient^[32]. Uric acid has been shown to exert pro-inflammatory and pro-oxidant effect both in adipose tissue and vascular smooth muscle cells^[33].

A recent largest observational population based cohort of non-diabetic American adult has suggested that uric acid might play a role in NAFLD^[33]. Sirota *et al*^[33] revealed that increased serum uric acid levels are associated with greater severity of NAFLD by ultrasonography. Several prospective and retrospective studies have also provided evidences supporting the association of high serum uric acid, the risk of T2DM, cardiovascular diseases and hepatitis^[34-36]. These have further supported that NAFLD is the hepatic manifestation of MetS.

It has been hypothesised that uric acid may contribute to insulin resistance by inducing local adipose tissue inflammation^[37]. This may reduced the production of adiponectin, a protein that is involved in glucose haemostasis^[37,38]. Furthermore, uric acid may stimulate hepatic lipogenesis, mediated by uric-acid dependent intracellular and mitochondrial oxidative stress^[33,37]. Whether such downstream effects of CPS1 and uric acid play a pathogenic role in NAFLD still requires further laboratory and clinical investigation.

In general, mitochondrial oxidative damage and impaired mitochondrial β -oxidation could contribute to hepatic steatosis. Subsequently, the progressive loss of mitochondrial function in conjunction with ER stress could play a key role in the transition of insulin resistance and NAFLD progression. However, little is known about the spectrum of changes of mitochondrial and ER function and their potential role in the natural history of NAFLD.

Acute phase proteins

An observational study in Japanese population study, high sensitivity C-reactive protein (hs-CRP) serum levels has been reported to be significantly higher in biopsy-proven NASH compared with SS^[39]. Furthermore, hs-CRP levels were significantly elevated in advanced NASH compared with those with mild NASH^[39]. However, the role hs-CRP in differentiating severity of NASH remains controversial. Zimmermann *et al*^[40] demonstrated a strong positive association between BMI and hs-CRP levels, which were measured with nephelometry, throughout the broad range of obesity. However, there were no significant associations between inflammation or fibrosis, respectively

and hs-CRP observed^[40].

Serum haemoglobin levels have been observed to be significantly elevated in extremely obese NAFLD patient and shown to be significantly associated with prevalence of NAFLD^[41-43]. Yu *et al*^[42] performed a serum protein fingerprint analysis, utilising the SELDI-TOF-MS technique combined with bioinformatic approaches and discovered that haemoglobin subunit alpha was significantly up-regulated in NAFLD group. It has been proposed that the relationship between serum haemoglobin and NAFLD may be partially modulated by haptoglobin (Hpt), an α 2-glycoprotein acute phase protein produced in response to an inflammatory insult^[44]. Serum haemoglobin levels may be a marker of hepatic steatosis but not of severity of NAFLD in obese patients.

Acute phase proteins are the key to the hepatic innate defences against insults and reduce hepatic tissue damage. Therefore, acute phase proteins may have an immediate role in hepatocytes injuries and could be used as NAFLD predictive markers in obese patients. However, the concentration of acute phase proteins does not reflect the severity of disease, suggesting different protein mechanism for NAFLD progression.

A recent study by Kamada *et al*^[44] has shed a light into the role of acute phase protein and progression of NAFLD. Kamada *et al*^[44] utilised lectin antibody enzyme-linked immunosorbent assay (ELISA) and demonstrated that serum fucosylated haptoglobin (Fuc-Hpt) underwent a stepwise increased with increasing hepatocyte ballooning scores in biopsy-proven NAFLD patients (AUROC ranging from 0.724 to 0.759). Fucosylated alpha-fetoproteins have been previously shown to be more selectively secreted into bile^[45]. Kamada *et al*^[44] hypothesised that an increase in ballooning hepatocytes could lead to the disruption of the fucosylation-based machinery and subsequently an increased secretion of Fuc-Hpt into the serum.

Pentraxin 3 (PTX-3), another acute-phase protein that resembles CRP in function and a possible modulator of complement system has been used as the marker for disease severity in cardiovascular, inflammatory and infectious diseases^[46]. Yoneda *et al*^[47] measured plasma PTX-3 levels with ELISA in biopsy-proven NAFLD, based on the method of Brunt. This study revealed a profound elevation of plasma PTX-3 levels in NASH and further suggested that PTX-3 could be a promising clinical marker for fibrosis severity in NASH^[47].

CRP and serum amyloid protein belong to the short pentraxins group, which have reported to be produced by the liver as a systemic response to local inflammation. On the other hand, PTX-3 is the prototypic long pentraxin, which is produced in damaged tissue and in response to proinflammatory stimuli such as interleukin 1 β (IL-1 β) and tumour necrosis factor- α (TNF- α). It has been anticipated that PTX-3 expression pattern may be more tissue specific than CRP^[47,48]. However, conflicting studies have been published regarding the association of PTX-3 levels, hs-CRP levels and the severity of Mets^[48]. Further

validation of PTX-3 is warranted to assess the usefulness of PTX-3 in understanding NAFLD.

Anti-inflammatory and anti-oxidants

A large cross-sectional study has demonstrated the inverse relationship between total serum bilirubin levels with vanadate oxidation method and the prevalence of NAFLD independent of known metabolic risk factors^[49]. Similar inverse relationship of NAFLD was discovered with conjugated and unconjugated bilirubin respectively^[50,51]. In addition, serum bilirubin has been long recognised to have an inverse relationship with the development of coronary artery disease^[52].

Bilirubin is the metabolic end product of heme catabolism as haem oxygenase-1 (HO-1), a stress-responsive protein that degrades the pro-oxidant heme to biliverdin. Subsequently, biliverdin reductase reduces biliverdin to bilirubin^[53]. The intrinsic amplification properties of biliverdin reductase redox cycle could readily augment the antioxidant effects of bilirubin by 10000-fold^[54]. The interesting challenge here is to link the clinical studies, which focus on serum levels of bilirubin with molecular mechanisms to understand the pathogenesis of NAFLD.

Recent cardiovascular research that focused on HO-1 activation in rat model of chronic heart failure has demonstrated significant improved survival due to the reduction in leukocyte activation and cardiomyocytes apoptosis. Furthermore, chronic HO-1 activation has shown to suppress the elevated levels of myeloperoxidase (MPO) activity, IL-1 β production and TNF- α production^[53]. Li *et al*^[55] characterised the expression of HO-1 in biopsy specimens of normal and active cirrhosis liver specimen with immunohistochemistry; and cultured human hepatic myofibroblasts with Northern and Western blot analysis. Li *et al*^[55] discovered that HO-1 can inhibit the proliferation of hepatic myofibroblasts. Therefore, it is anticipated that bilirubin can inhibit the pathogenesis of NAFLD and NASH through the potent antioxidant, anti-inflammatory and anti-fibrogenic effects. Further research is needed to elucidate the mechanisms underlying this association and to establish the role of serum bilirubin as a potential protective marker for NAFLD risk.

Extracellular matrix

While the presence of inflammation is due to lipid accumulation, an imbalance of ECM production and degradation will therefore lead to fibrogenesis. The association of serum concentration of ECM components, especially increased serum hyaluronic acid, type IV collagen 7S and laminin levels measured with routine laboratory parameters and the degree of fibrosis in NAFLD have been studied extensively^[56-59]. Hyaluronic acid is a component of ECM, which synthesised by mesenchymal cells. The increased production and delayed clearance of hyaluronic acid have been demonstrated to be associated with the severity of hepatic inflammation^[56]. Type IV collagen is the main collagen component of basement membrane whereas laminin is the main non-collagenous glycopro-

tein of the ECM that deposited in the space of Disse during sinusoidal capillarisation^[58,60]. It is believed that hepatocytes ballooning and releases of type IV collagen and laminin are the main causes of increased their serum levels. However, the mechanism of this interaction is still unknown.

It is important to note that hyaluronic acid, type IV collagen 7S and laminin have been shown to increased in cases of fibrosis resulting from alcoholic, primary biliary cirrhosis and chronic viral hepatitis^[56,61,62]. Therefore, hyaluronic acid, type IV collagen 7S and laminin may be useful in detecting the presence of severe fibrosis in chronic liver disease, but not specific to NAFLD.

Lumican, a highly biologically active 40-kDa keratin sulfate proteoglycan member of the leucine-rich repeat protein superfamily, is known to be progressively over-expressed with the increasing severity of NAFLD and NASH^[63,64]. Similar to the liver, the maintenance of myocardial ECM and regulation of fibrotic myocardial remodelling are essential for normal cardiac physiological function^[65]. A recent cardiac failure research discovered that lumican production is induced by a combination of inflammation and mechanical stimuli from cardiac fibroblasts^[66]. Further laboratory research also revealed that in the absence of lumican, hepatocytes are protected against fibrosis despite ongoing injury^[64]. Therefore, lumican might have an important role in tissue remodelling and contribute to fibrogenesis.

ECM degradation occurs predominantly as a consequence of the action of MMPs^[67]. MMPs has been known to be an important regulatory enzyme in inflammatory process to modulate leukocyte influx, either through regulation of barrier function, cytokine or chemokine activity, or gradient formation^[68]. An up-regulation of tissue inhibitors of metalloproteinases (TIMPs) in liver fibrosis may impair MMPs' activity and lead to altered ECM production^[69]. Interestingly, a growing body of evidences has demonstrated MMPs' role in liver fibrogenesis^[67,69]. Serum MMP-1 level assayed by ELISA has been found to be negatively correlates with the severity of fibrosis whereas MMP-1 mRNA quantified with spectrophotometer and single reverse transcription polymerase chain reaction (PCR) has been found to be elevated in liver tissue as fibrosis progressed^[70,71]. The decreased synthesis of MMP-1 is believed to due to HSC apoptosis or necrosis. However, the link between increased hepatic MMP-1 expression and decreased serum MMP-1 remained unanswered.

Notable increase in serum MMP-9 level with ELISA was found in NASH and hepatitis C infected patients^[72]. Furthermore, immunohistology of the liver specimen and revealed a striking difference of the immunolabelling patterns between NASH and hepatitis C infected liver^[72]. Hepatitis C infected liver demonstrated an essentially localised labelling in the cytoplasm of hepatocytes, biliary canaliculi and bile ducts whereas NASH liver demonstrated a discrete labeling of neutrophil subpopulation^[72]. The difference of MMP-9 patterns suggested a different

pathophysiological involvement of this protease in the fibrogenesis underlying different etiology.

On the other hand, Wanninger *et al.*^[67] utilised sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analysis in primary human hepatocytes, steatosis human hepatocytes and murine NASH hepatocytes to study the activity of MMP-9. Wanninger *et al.*^[67] concluded that MMP-9 activity is associated with hepatic inflammation and fibrosis but not with hepatic steatosis and body weight. This raises the question of peripheral adipocytes involvement with other inflammatory mediators that could have contributed to the progression of NAFLD and NASH. Furthermore, Wanninger *et al.*^[67] revealed a negative correlation of MMP-9 activity with serum adiponectin in murine NASH models. This finding highlighted the role of inflammation in the progression of NAFLD. The anti-inflammatory activity of adiponectin will be further discussed in the genomic section.

A study utilising reverse transcription and quantitative real-time reverse transcription PCR in animal models has demonstrated an accumulation of macrophages and increased gene expression of MMP-12, MMP-13 and TIMP-1 in predominantly adipose tissue compared with liver tissue^[73]. The author proposed that attenuated hepatic MMP expression in both livers and adipose tissue might be responsible for the shifting balance of fibrogenesis^[73]. However, the key source of these enzymes and tight relationship between adipose tissue dysfunction and developments of hepatic fibrosis remain unclear.

Despite the growing understanding of MMPs and TIMPs, the conclusive role of each MMPs and TIMPs subtypes in appropriate human model is still limited. However, this review highlighted the increased recognition of liver fibrosis as dynamic pathological process that involves both progression and regression of ECM.

Immune cells and cytokines

A paper by Zhan and An has comprehensively reviewed the role of liver innate immune cells such as T helper cells (Th cells), activated kupffer cells (KC) and natural killer T (NKT) cells in the development of NAFLD^[74]. Hereby, we further reviewed related cytokine papers that being published later.

It has been long proposed that it was Th1 cytokine excessive production in NAFLD results in hepatic insulin resistance and NASH^[74]. However, a recent laboratory research on C57BL/6 and Balb/c mice with mRNA extraction and real-time PCR demonstrated that imbalance between Th1 and Th2 did not account for the increased NASH severity and suggested that macrophage responses are the important contributors to NAFLD progression^[75]. In addition, cytokines are not only being produced by local sources but also by peripheral blood cells. The presence of an altered phenotype and functionality of circulating immune cells has been speculated to be a distinctive characteristic in NASH patients^[76,77].

Innate immune cells have the ability to secrete cyto-

kines and to promote influx of inflammatory cells. To date, emerging strong evidences suggested that cytokines such as TNF- α and IL-1 are sensitising factors acting upon leukocyte infiltration of the liver^[76,78]. These cytokines and chemokines effects contributed to intracellular oxidative stress and mitochondrial dysfunction, which amplified hepatocytes damage. The cytokines mechanism appears to be like a vicious cycle. The further disrupted cellular mitochondrial respiratory chain can thereby cause the formation reactive oxidative species of nearby hepatocytes^[76]. As a consequence, HSCs is activated and lead to hepatic fibrosis. The development and progression of NASH act in a cooperative manner with pro-inflammatory cytokines.

IL-6 is a pro-inflammatory cytokine that has been studied extensively for its wide range of biological function. IL-6 has been proposed to have direct and indirect deleterious role such as induction of inflammation, hepatoprotector, regulators of acute phase response and insulin signalling^[79]. Wieckowska *et al*^[80] demonstrated a markedly increased hepatic IL-6 expression assayed with immunohistochemistry in patients with NASH as compared to SS or normal liver. The hepatic IL-6 expression in this study cohort has showed to be correlates with the severity of inflammation and fibrosis^[80]. At the same time, a positive correlation was observed between the plasma IL-6 levels measured with ELISA and hepatic IL-6 expression^[80]. However, discrepant results have been reported regarding IL-6 in NAFLD studies. Haukeland *et al*^[81] determined IL-6 in 47 biopsy-proven NAFLD and 30 controls with enzyme immunoassays and revealed no significant changes in NASH group compared to SS.

Interestingly, Haukeland *et al*^[81] further evaluated serum level of CC-chemokine ligand 2 /monocyte chemoattractant protein-1 (CCL2/MCP1) and demonstrated an increasing levels from healthy controls to SS and reached the highest levels in NASH. It has been proposed that an increased circulating CCL2/MCP-1 levels may be related to the development of NASH^[79,81]. CCL2, an acute and chronic phase chemokine has been known to be secreted by HSCs. CCL2 is responsible for monocyte and macrophage infiltration of liver and maintaining hepatic inflammation and fibrogenesis^[82]. On the other hand, MCP-1 is known to be derived from visceral adipose tissue and reached liver *via* portal circulations^[83]. Many more studies are still warranted to understand the pivotal roles of CCL2 and MCP-1 in the development of inflammatory responses in the liver. The lipid accumulation from visceral tissue and hepatocellular could significantly contribute to the expression of CCL2 and MCP-1. Therefore, the concentrations of both chemokines are crucial for the recruitment of immune cells to sites of inflammation.

It is believed that adipose tissue-derived cytokine could also affects liver innate immune cells^[74]. With LFQP approach, Bell *et al*^[18] demonstrated that retinol binding protein 4 (RBP4), an adipokine that associated with insulin resistance and T2DM was significantly decreased in expression with increasing NAFLD severity.

Utilising ELISA, Alkhoury *et al*^[84] also demonstrated an inverse relationship between RBP4 and liver fibrosis. However, there were also contrary reports that demonstrated significantly elevated serum RBP4 in NAFLD patients^[85,86]. The lack of consistency prompted us to re-evaluate RBP4 as a potential novel marker to assess the progression of fibrogenesis in NAFLD.

On the other hand, the link between RBP4 and T2DM are more obvious. Graham *et al*^[87] revealed an increased serum RBP4 with ELISA in patients with T2DM and a decreased expression of glucose transporter 4 (GLUT4). The mechanism behind this remains unclear, but raises the possibility of adipocytes involvement in glucose sensing. A nicely summarised review article by Christou *et al*^[88] indicated that RBP4 might have the ability to induce insulin resistance in an autocrine or paracrine mechanism in the adipose tissue. It is noteworthy that circulating RBP4 can be influenced by non-metabolic condition such as renal failure and intervention such as anti-diabetic drugs and exercise^[88,89]. Therefore, the association of RBP4 level and MetS could be affected and should be interpret with caution. The pathogenic role of RBP4 has contributed to genetic studies of RBP4 locus in human chromosome 10q, which have been linked to hyperinsulinemia or early onset T2DM^[89]. The polymorphism of *RBP4* gene polymorphism has been linked with the development of T2DM and obesity in different populations^[89,90]. However, the associations among RBP4 polymorphism, serum RBP4 levels and measures of MetS have not been universally described^[90].

GENOMIC STUDIES

The first GWA study was performed by Romeo *et al*^[91] using a phenotype based on magnetic resonance spectroscopy. The authors identified that an allele in human patatin-like phospholipase domain containing 3 gene (*PNPLA3*) (rs738409; I148M) was strongly associated with increased hepatic lipids levels and hepatic inflammation. This study importantly raised that genetic factors could account towards the susceptibility of NAFLD^[91]. With regards to the pathophysiology of NAFLD, variations in genes involved in hepatic lipid metabolism, ECM balance, cytokines and insulin resistance will be discussed in this review.

Genes that affect lipolysis

Polymorphisms of *PNPLA3* gene have been found to be strongly associated with the pathogenic status of NAFLD in different populations^[91-93]. *PNPLA3* gene, encoding adiponutrin is known to have lipase activity *in vitro* and has been found to be involved in glucose and lipid metabolism^[91]. Despite several publications on the association between *PNPLA3* polymorphisms and NAFLD, the physiological functions of the *PNPLA3* still remain unclear.

A well-conducted meta-analysis revealed that GG genotype of *PNPLA3* (rs738409; I148M) exerts a stronger effect on hepatic lipids accumulations and patients

with GG phenotype are more susceptible to aggressive disease associated with higher fibrosis scores than CC genotype^[94]. Furthermore, Sookoian *et al*^[94] found no correlation between increased liver disease severity and heterozygous or homozygous mutants of G-allele.

The link between NAFLD stage and PNPLA3 is well established, but the association of insulin resistance and obesity with PNPLA3 are less clear^[95,96]. A recent review of *PNPLA3* polymorphism suggested that PNPLA3 is more likely to influence lipid content and liver disease severity but not insulin resistance^[95]. Lallukka *et al*^[97] demonstrated that NAFLD patients with polymorphism of PNPLA3 is not characterised by adipose tissue inflammation, insulin resistance and features of the metabolic syndrome such as hyperinsulinaemia, hypertriglycerolaemia and a low HDL-cholesterol concentration. Despite the less clear relationship in insulin resistance, the severity of NAFLD has been proven to be related to the PNPLA3 in T2DM populations. Therefore, screening analysis of the *PNPLA3* polymorphism could still be useful to select T2DM patients who need specific interventions as prevention for NAFLD^[98].

A recent transplant research revealed that PNPLA3 of non-CC genotype is associated with post-transplant obesity but not independently with diabetes. Furthermore, the donor genotypes were not associated with obesity or diabetes^[99]. This research highlighted the potential of peripheral mechanism for insulin resistance and could reflect a multi-factorial gene involvement in NAFLD. One point to note is that the effect on allograft steatosis was not studied.

Ample evidences exist in the literatures that triglycerides (TG) accumulation in liver is a result of obesity and diabetes^[100]. Surprisingly, an accumulation of TG has been suggested to be a protective mechanism to prevent progressive liver damage in NAFLD^[101]. Adipose tissue triglyceride lipase (ATGL) has been found to be a rate-limiting enzyme for degradation of TG and has been implicated in the pathogenesis and progression of NAFLD. A deficiency of ATGL was proposed to lead to TG accumulation in many organs, including the liver^[102,103]. In murine model, ATGL knock out (ATGL-KO) mice demonstrated increased hepatic lipids accumulation during induced-ER stress but suppressed gene expression of inflammatory markers. Further evaluation suggested that an enrichment of oleic acid in the TG pool plays the main role for ATGL in protection from potentially harmful consequences of ER stress^[103]. Furthermore, ATGL-KO animal model demonstrated a high insulin sensitivity despite liver TG storage^[102]. Therefore, the progressions of NAFLD in obese and diabetes patients have a distinguishable phenotypic imprint on the hepatocyte lipidome. Further research in ATGL polymorphism and lipidome could potential elucidates the lipolytic catabolism pathway in NAFLD.

Apolipoprotein C3 (ApoC3) is the most important components of very low density lipoprotein (VLDL). ApoC3, also a strong inhibitor lipoprotein lipase (LPL)

has been found to enhance fatty acid uptake from plasma TG in adipose tissue in mice^[104]. Therefore, a deficiency of ApoC3 could leads to higher susceptibility to diet-induced obesity followed by more severe development of insulin resistance^[104].

Approximately between 50% and 75% of patients with T2DM have fatty liver diagnosed by ultrasound^[4]. Insulin resistance has been anticipated to play a major role in the pathogenesis of T2DM and may enhance hepatic fat accumulations by increasing free fatty transportation due to the effect of hyperinsulinemia to stimulate anabolic process^[4,105]. Petersen *et al*^[105] demonstrated a 3- to 4-fold increased in prevalence of insulin resistance in Asian-Indian men compared with Caucasian men. Later, Petersen *et al*^[106] further demonstrated that the polymorphisms of ApoC3 (C-482T and T-455C) are associated with NAFLD and insulin resistance in Indian men. However, there is a contrary report suggested that the genetic variants in ApoC3 does not contribute to NAFLD in Finnish populations^[107]. The reason regarding the divergent results remains unknown but importantly highlighted the ethnic differences in disease susceptibility.

Given the essential contribution of lipogenesis pathway, another common gene variant, glucokinase regulatory protein (*GCKR*) gene has been studied extensively^[108-110]. *GCKR* regulates glucokinase, a phosphorylating enzyme that responsible for hepatic glucose metabolism and activates hepatic lipogenesis^[108]. Polymorphism of *GCKR* was found to be significantly associated with increased risk of T2DM, particularly in Asian population^[109,110]. Furthermore, polymorphism of *GCKR* (rs780094 and rs1260326) has been shown to be associated with NAFLD in Chinese people and obese youth respectively^[108,111]. In addition, Tan *et al*^[112] further suggested that the interactions of *GCKR* and *PNPLA3* genes can increase susceptibility to NAFLD.

The protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha PGC-1 α , encoded by the *PPARGC1A* gene, has also been recognised to be the transcriptional co-activator of peroxisome proliferator activated receptor gamma (*PPAR- γ*) gene^[113]. The PGC-1 α and *PPAR- γ* pathway play a central role in energy metabolism, mitochondrial biogenesis and function, oxidative phosphorylation, gluconeogenesis and lipogenesis and induction of apoptosis^[113,114]. *PPARGC1A* knock out mice have been reported to develop hepatic steatosis, as a result of the combination of reduced mitochondrial respiratory capacity and increased expression of lipogenic genes^[115]. However, the role of PCG-1 α in MetS is less clear than *PPAR- γ* .

Controversial reports exist in the literatures, regarding *PPARGC1A* gene in patient's susceptibility for NAFLD. Hui *et al*^[116] demonstrated that only polymorphism of *PPAR- γ* (C161T) are associated with NAFLD susceptibility possibly through the adiponectin pathway, not *PPARGC1A* gene. Subsequent reports demonstrated that *PPARGC1A* (rs8192678) risk A allele is associated with an increased risk of NAFLD and is independent of the

effect of the *PNPLA3* (rs738409) polymorphism in Taiwanese obese children population^[117]. The role of *PCG-1α* remains unclear but has been hypothesised to be associated with exercise^[118]. On the other hand, the genetic variation in *PPAR-γ* (Pro12Ala and C1431T) has been studied extensively in different ethnic cohorts^[119-121]. Polymorphism of *PPAR-γ* has been concluded to be strongly associated with NAFLD whereas polymorphism of G allele in *PPAR-γ* gene (rs10865710) is only associated with lobular inflammation^[119].

Genes that affect adipokines

Adipocytes play multiple endocrine roles in the body. As body mass index (BMI) increases, the metabolic role of adipocytes changes^[122]. An increased understanding of the role of the adipocyte and its associated adipokines could give us some clues in understanding MetS and NAFLD.

Adiponectin is the most abundant and adipose-specific adipokine. Contrary to other adipokines, adiponectin correlates negatively with BMI, insulin resistance, plasma TG, LDL-cholesterol, hepatic fat content and progression to NASH in NAFLD patients^[38,123]. Adiponectin has been reported to be higher in women and in Caucasian compared with Asian or African individuals^[123,124].

Adiponectin is known to have insulin-sensitising, antifibrogenic and anti-inflammatory properties. Studies have found that Adiponectin is involved in the activation of AMPK and downregulates the expression of sterol regulatory element-binding protein 1c (SREBP1c), a transcription factor that regulates cholesterol and lipid synthesis. As a result, this lead to decreased gluconeogenesis, decreased free fatty acid influx into the liver, increased free fatty acid oxidation and decreased *de novo* lipogenesis^[38,125]. Two common SNPs in Adiponectin gene (45GT and 276GT) have been found to be significantly associated with the presence of NAFLD in non-obese, non-diabetic and normolipidemic patients and were subsequently correlated to the severity of NASH. Furthermore, Adiponectin gene (45GT and 276GT) has been proven to be the acute modulation of postprandial adiponectin levels and lipoprotein responses after fat ingestion^[126].

However, controversy exists in the literatures. Some authors have reported a lack of association between Adiponectin gene and NAFLD^[38,127]. Furthermore, several studies demonstrated a weak association between adiponectin gene polymorphism and cardiovascular disease^[128,129]. The association between adiponectin polymorphisms and T2DM has been inconsistent and recent meta-analysis concluded that adiponectin gene polymorphism does not influence the development of T2DM^[130,131]. Interestingly, two recent meta-analyses have revealed the potential protective factor of Adiponectin gene of different allele for cancer risk^[132,133]. One point to note was that these meta-analyses were not specific to liver aetiology. However, recent evidences demonstrated a correlation between NAFLD and colorectal cancer^[134]. The mechanism behind this remains unknown but raises

the possibility of Adiponectin's anti-inflammatory effect that suppress TNF- α and other proinflammatory cytokines^[38]. Further research is warranted to elucidate the link between cytokines and progression of NAFLD.

Leptin (LEP), an adipocyte-derived hormone exerts its action through leptin receptor (LEPR), a single-transmembrane-domain receptor of the cytokine-receptor family has been shown to be responsible for satiety and energy homeostasis^[135,136]. *LEP* and *LEPR* mutations have been shown to be strongly associated with obesity and endocrine dysfunction^[135,137]. In study of NAFLD, Leptin-deficient *ob/ob* mouse has demonstrated a dramatic up-regulations of hepatic *de novo* lipogenesis markers and a significant impairment in hepatic mitochondrial function^[138]. Recently, an association between variants of *LEPR* and *PNPLA3* has been reported. The interactions between *LEPR* and *PNPLA3* genes showed an increased risk of NAFLD compared to either gene alone^[139].

Genes that affect cytokines production

Little is known about the evolvement of NAFLD, a relative benign condition to NASH, an irreversible inflamed stage of liver disease. Inflammatory cytokines has been proposed to play a central role in the progression of NAFLD^[140]. Cytokines are central mediators of hepatic inflammation, cells apoptosis and cells regeneration. TNF- α and IL-6 have been found to be the initial cytokines chemokines that being produced after hepatic insults^[140,141].

TNF- α has been known to interact between fat accumulation and hepatic inflammation^[141]. Coulon *et al*^[141] suggested that TNF- α was significantly higher in NASH patients compared to SS patients. This highlighted the potential role of TNF- α in NASH. Furthermore, a recent meta-analysis has demonstrated that polymorphisms of TNF- α (-238) is particularly associated with the susceptibility to NAFLD^[142]. TNF- α has also been previously known to be an important cytokine that regulates insulin resistance by affecting insulin receptor substrate-1 (IRS-1) and insulin receptor kinase (IRK) in the insulin signal transduction pathway^[142]. Nevertheless, the involvement of TNF- α in insulin resistance is debatable. Polymorphisms in gene such as IRS-1 (972 Arg) and ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1) that directly affect insulin receptor activity have demonstrated that insulin resistance does contribute to the progression of NAFLD^[143]. However, a recent study by Aparicio-Vergara *et al*^[144] demonstrated a disassociation of TNF- α induced hepatic inflammation and insulin resistance.

IL-6 is another cytokine that involved in the regulation of several cellular processes and has been demonstrated to be associated with NASH in several ethnic cohorts^[141,145]. However, the polymorphism of G/C allele is questionable. Carulli *et al*^[146] demonstrated that polymorphism of IL-6 -174C is more prevalent in NAFLD and associated with insulin resistance while other studies demonstrated that polymorphism of C allele was unlikely

Table 1 Proteomic studies of non-alcoholic fatty liver disease

Protein categories	Protein markers	Ref.	Year	Findings	Implications
Protein carrier	Apolipoproteins	Choe <i>et al</i> ^[21]	2013	Independent association between ApoB/ApoA1 ratio and NAFLD	NAFLD is the hepatic manifestation of MetS
	CD5L	Gray <i>et al</i> ^[22]	2009	Identified a pattern of serum apolipoproteins and CD5L to distinguish between pre-cirrhotic NAFLD and cirrhotic NAFLD	ApoB/ApoA1 ratio as a predictable marker of cardiovascular risk in NAFLD Possibility of different trigger factors for the progression of NAFLD, NASH to HCC
Metabolic pathways	CPS1	Rodríguez-Suárez <i>et al</i> ^[24]	2010	Identified CD5L as poor biomarkers for HCC Decreased in NASH compared to control patients	Down-regulation of CPS1 may lead to an increase of uric acid ^[31]
	GRP78	Rodríguez-Suárez <i>et al</i> ^[24]	2010	Decreased in NASH compared to control patients	Increased <i>de novo</i> lipogenesis might play a role in NAFLD pathogenesis ^[27]
	Uric Acid	Sirota <i>et al</i> ^[33]	2013	Association of increased serum uric acid level and severity of NAFLD	Mitochondrial oxidative damage could contribute to hepatic steatosis
Acute phase protein	hs-CRP	Yoneda <i>et al</i> ^[39]	2007	Increased hs-CRP serum level in advanced NASH	Acute phase proteins may have an immediate role in hepatocytes injuries
	Haemoglobin	Yu <i>et al</i> ^[42]	2012	Association of elevated serum haemoglobin levels and prevalence of NAFLD	predictive markers
	Fuc-Hpt	Kamada <i>et al</i> ^[44]	2013	Stepwise increased with increasing hepatocyte ballooning scores in biopsy-proven NAFLD patients	Serum levels of acute phase proteins do not necessary reflect the severity of disease
	PTX-3	Yoneda <i>et al</i> ^[47]	2008	A profound elevation of plasma PTX-3 level in NASH patients	NAFLD progression has a more complex underlying protein mechanism
Anti-inflammatory and anti-oxidant	Bilirubin	Kwak <i>et al</i> ^[49]	2012	An inverse relationship between total serum bilirubin level and the prevalence of NAFLD	Bilirubin has antioxidant, anti-inflammatory and anti-fibrogenic effects
Extracellular matrix	Hyaluronic acid	Kaneda <i>et al</i> ^[56]	2006	Association of increased serum concentration of ECM components and degree of fibrosis in NAFLD	An imbalance of ECM production and degradation could lead to fibrogenesis, but not specific to NAFLD
	Type IV collagen 7S	Yoneda <i>et al</i> ^[59]	2007		
	Laminin	Gabrielli <i>et al</i> ^[60]	1996		
	Lumican	Krishnan <i>et al</i> ^[64]	2012	Over-expressed of Lumican with increasing severity of NAFLD and NASH	Unregulated tissue remodelling and fibrogenesis could contribute to the progression of NAFLD
	MMP-9	D'Amico <i>et al</i> ^[72]	2010	An increase in serum MMP-9 levels in NASH and hepatitis C infected patients Demonstrated a difference of immunolabelling patterns between NASH and hepatitis C infected liver	Possibility of a different pathophysiological involvement of protease in the fibrogenesis of different liver etiology
Immune cells and cytokines	CCL2/MCP1	Haukeland <i>et al</i> ^[81]	2006	Demonstrated an increasing level of CCL2/MCP1, from healthy controls to NASH	The lipid accumulation from visceral tissue and hepatocellular could be responsible for the recruitment of immune cells
	RBP4	Bell <i>et al</i> ^[18]	2010	A decreased expression of RBP4 with increasing NAFLD severity	RBP4 could contribute to the fibrogenesis pathway in NAFLD
		Alkhouiri <i>et al</i> ^[84]	2009	An inverse relationship between RBP4 levels and liver fibrosis	
		Graham <i>et al</i> ^[87]	2006	Association of increased serum RBP4 with T2DM and a decreased expression of glucose transporter 4	RBP4 is associated with insulin resistance and T2DM
		Christou <i>et al</i> ^[88]	2012	RBP4 has the ability to induce insulin resistance in the adipose tissue Circulating RBP4 can be influenced by non-metabolic conditions and interventions	The association of RBP4 level and MetS is still not conclusive and should be interpret with caution

NAFLD: Non-alcoholic fatty liver disease; MetS: Metabolic syndrome; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma; CPS1: Carbamoyl phosphate synthase 1; hs-CRP: High sensitivity C-reactive protein; PTX-3: Pentraxin 3; ECM: Extracellular matrix; MMP: Matrix metalloproteinases; CCL2/MCP1: CC-chemokine ligand 2/monocyte chemo-attractant protein-1; T2DM: Type II diabetes mellitus.

Table 2 Genomic studies of non-alcoholic fatty liver disease

Gene categories	Genes	Ref.	Year	Findings	Implications
Gene that affect lipolysis	PNPLA3	Romeo <i>et al</i> ^[91]	2008	PNPLA3 polymorphism is strongly associated with the pathogenic status of NAFLD in different populations	A well established link between PNPLA3 and NAFLD progression
		Sookoian <i>et al</i> ^[94]	2011	GG genotype of PNPLA3 (rs738409; I148M) exerts a stronger effect on hepatic lipids accumulations	Identified the patients group who is more susceptible to aggressive diseases
		Dubuquoy <i>et al</i> ^[95]	2013	PNPLA3 polymorphism is more likely to influence lipid content and liver disease severity but not insulin resistance	A less clear association of insulin resistance with PNPLA3 polymorphism
	ATGL	Chitraju <i>et al</i> ^[102]	2013	Demonstrated that ATGL-KO animal models have high insulin sensitivity	An accumulation of TG could be a protective mechanism in NAFLD
	ApoC3	Petersen <i>et al</i> ^[106]	2010	ApoC3 polymorphism is associated with NAFLD and insulin resistance in Indian populations	Highlighted the ethnic differences in ApoC3 polymorphism and disease susceptibility
		Hyysalo <i>et al</i> ^[107]	2012	ApoC3 polymorphism does not associated with NAFLD in Finnish populations	
	GCKR	Santora <i>et al</i> ^[108]	2012	GCKR polymorphism is associated with NAFLD	Lipogenesis pathway plays a role in NAFLD pathogenesis
		Tan <i>et al</i> ^[112]	2013	Interactions of GCKR and PNPLA3 genes can increase susceptibility to NAFLD	
	PPARGC1A and PPAR-γ	Hui <i>et al</i> ^[116]	2008	PPARGC1A gene polymorphism is not associated with NAFLD	The PGC-1α and PPAR-γ pathway play a role in NAFLD progression
		Gawrieh <i>et al</i> ^[119]	2012	PPAR-γ polymorphism is associated with NAFLD	The role of PGC-1α (encoded by the PPARGC1A gene) in MetS is less clear than PPAR-γ
Genes that affect adipokines	Adiponectin	Musso <i>et al</i> ^[126]	2008	Adiponectin gene polymorphism is associated with the presence of NAFLD	<i>De novo</i> lipogenesis plays a role in NAFLD
		Han <i>et al</i> ^[130]	2011	Adiponectin gene polymorphism does not influence the development of T2DM	Possibility of different mechanisms for insulin sensitising in NAFLD
		Li ^[20]	2012	Demonstrated a correlation between NAFLD and colorectal cancer	Raises the possibility and association of anti-inflammatory effect in NAFLD progression and oncological disease
	LEP and LEPR	Zain <i>et al</i> ^[139]	2013	Demonstrated an association between variants of LEPR and PNPLA3	Patients with mutations of both LEPR and PNPLA3 are highly susceptible to NAFLD
Genes that affect cytokines	TNF-α	Wang <i>et al</i> ^[142]	2012	Polymorphisms of TNF-α are associated with the susceptibility to NAFLD	Insulin resistance and hepatic inflammation are related
	IL-6	Carulli <i>et al</i> ^[146]	2009	Polymorphisms of IL-6 are more prevalent in NAFLD and associated with insulin resistance	Insulin signal transduction pathway does contribute to the progression of NAFLD
		Giannitrapani <i>et al</i> ^[145]	2013	Polymorphisms of IL-6 are unlikely to associate with insulin resistance	

NAFLD: Non-alcoholic fatty liver disease; MetS: Metabolic syndrome; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma; CPS1: Carbamoyl phosphate synthase 1; hs-CRP: High sensitivity C-reactive protein; PTX-3: Pentraxin 3; ECM: Extracellular matrix; MMP: Matrix metalloproteinases; CCL2/MCP1: CC-chemokine ligand 2/monocyte chemo-attractant protein-1; T2DM: Type II diabetes mellitus; PPAR-γ: Peroxisome proliferator activated receptor gamma.

to associate with insulin resistance^[145]. Hence, insulin resistance and augmentation of hepatic inflammation could be modulated by other factors than TNF-α and IL-6.

CURRENT KNOWLEDGE

There is no doubt that there is a complex interplay between the body environment, predispose genetics and external environment (diet) for the development and progression of NAFLD. From the literatures, we know that NAFLD is characterised by insulin resistance, metabolic hyperlipidaemia and hepatic inflammation (Tables 1 and 2). Insulin resistance, accumulation of lipids (both visceral and hepatocellular) and persistent hepatic inflamma-

tion can result in a complex biochemical interaction. This could alter the cytokine profile and cell biology. A resultant disequilibrium in lipid homeostasis leads to hepatocellular lipid accumulation and an increase in oxidative stress due to mitochondrial dysfunction. Many studies have shed light on the role inflammation in progression of NAFLD to NASH. The activation of cell death pathways such as apoptosis and necrosis has redundant roles in further triggering liver damage and fibrosis.

One of the crucial aspects from this review is that promising anti-inflammatory and innate immune mediators such as bilirubin, TNF-α and IL-6 are also commonly associated with cardiovascular disease, obesity and diabetes mellitus. The background of MetS further con-

finds these shared observations. An increased understanding of the roles of the liver in metabolic diseases or *vice versa* could lead to development of improved targeted strategies for disease prevention and treatment.

This review also highlighted a considerable disease variation exists in the prevalence and severity of NAFLD. Therefore, suggesting that the risk of morbidity and mortality that might be influenced by a combination of genetic and environmental factors, which has strongly proven by the *PNPLA3* polymorphisms. The improve understanding and further identification of genetic polymorphisms could lead to two potential applications for clinical practice. Firstly, genetic biomarkers could allow early disease diagnosis of higher risk individuals or family. Secondly, genetic biomarkers could potentially lead to the development of novel preventive and targeting measures of NAFLD.

CONCLUSION

Advances in proteomic technologies have contributed to the discovery of clinically important protein biomarkers, which are the key molecules that reflect biological reactions. Extensive literature searches revealed several potential serum protein markers that could differentiate NAFLD and NASH. Further validations of these proteins markers in larger cohorts are required to reflect the degree of hepatic fibrosis. In addition, genomic studies have further revealed the importance of genetic polymorphisms in various steps of the pathogenesis of NAFLD. Clearly, unfavourable genetic polymorphism coupled with unfavourable biological environment can increase a patient's susceptibility to the development of NAFLD and its progression to NASH. In general, further breakthrough and investigation of changes in protein expression levels are still warranted to understand the pathophysiology of NAFLD.

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