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Review

Genetic modifiers of non-alcoholic fatty liver disease progression

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is now recognised as the most common cause of liver dysfunction worldwide. However, whilst the majority of individuals who exhibit features of the metabolic syndrome including obesity and insulin resistance will develop steatosis, only a minority progress to steatohepatitis, fibrosis and cirrhosis. Subtle inter-patient genetic variations and environment interact to determine disease phenotype and influence progression. A decade after the sequencing of the human genome, the comprehensive study of genomic variation offers new insights into the modifier genes, pathogenic mechanisms and is beginning to suggest novel therapeutic targets. We review the current status of the field with particular focus on advances from recent genome-wide association studies.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disease encompassing simple fatty infiltration of the liver parenchyma (steatosis), fat and inflammation (non-alcoholic steatohepatitis; NASH) and cirrhosis, in the absence of excessive alcohol consumption (typically a threshold of <20 g/day for women and <30 g/day for men is adopted) [1,2]. Population studies show that NAFLD is strongly associated with obesity, insulin resistance/type II diabetes mellitus and dyslipidaemia and it is considered by many to be the hepatic manifestation of the metabolic syndrome [3–5]. It is now recognised that NAFLD is the most common cause of liver dysfunction in developed countries [1]. Estimates vary between populations however one large European study found NAFLD present in 94% of obese patients (BMI >30 kg/m^2), 67% of overweight patients (BMI >25 kg/m^2), and 25% of normal weight patients [6]. The overall prevalence of NAFLD in type 2 diabetics ranges from 40% to 70% [6]. As only a minority of patients with NAFLD progress to more advanced disease characterised by inflammation, fibrosis, cirrhosis and hepatocellular carcinoma (HCC), NAFLD is best considered a complex disease trait where subtle inter-patient genetic variations and environment interact to determine disease phenotype and progression [7,8]. Here we will review the current understanding of genetic modifiers of NAFLD/NASH with particular focus on data from human studies (Fig. 1).

2. Pathogenesis

Our current understanding of disease pathogenesis has been achieved through clinic based research and the translational study of specific animal models [9,10]. The initiating events in NAFLD/NASH relate to the development of obesity and insulin resistance. Together, these promote hepatic free fatty acid (FFA) flux which provides the appropriate milieu for NAFLD/NASH to develop. Importantly, the visible steatosis that has for some time been considered the 'first hit' in the pathogenesis of NAFLD/NASH is now recognised by many investigators to be an epiphenomenon reflecting these changes in hepatocyte FFA flux and associated cellular stress responses. Recognition of this means that steatosis should now be considered an early adaptive response to hepatocyte stress through which potentially lipotoxic FFAs are partitioned into relatively stable intracellular triglyceride stores. This was elegantly demonstrated by silencing of hepatic *DGAT2* expression, a key enzyme mediating the conversion of FFA to triglyceride [11]. Rather than ameliorating steatohepatitis, the consequent reduction in hepatocyte triglyceride synthesis was associated with a greater level of fatty acid oxidation, particularly through Cyp2E1, leading to greater oxidative stress, cellular damage and higher serum transaminase levels [11]. Discussion of pathogenesis and the genetic modifiers of progressive NAFLD should now consider the combined effects of several fundamental biochemical and immunological mechanisms of liver injury rather than adhering to a sequential 'two-hit' paradigm. These effects include: (1) Direct hepatocyte lipotoxicity; (2) Hepatocellular oxidative stress secondary to free radicals produced during β- and ω-FFA oxidation; (3) Endotoxin/TLR4 induced Kupffer cell cytokine release; (4) Cytokine release; and (5) Endoplasmic reticulum (ER) stress. Consequent cellular damage triggers a mixture of immune mediated hepatocellular injury and both necrotic and apoptotic cell death pathways [12–14]. If these

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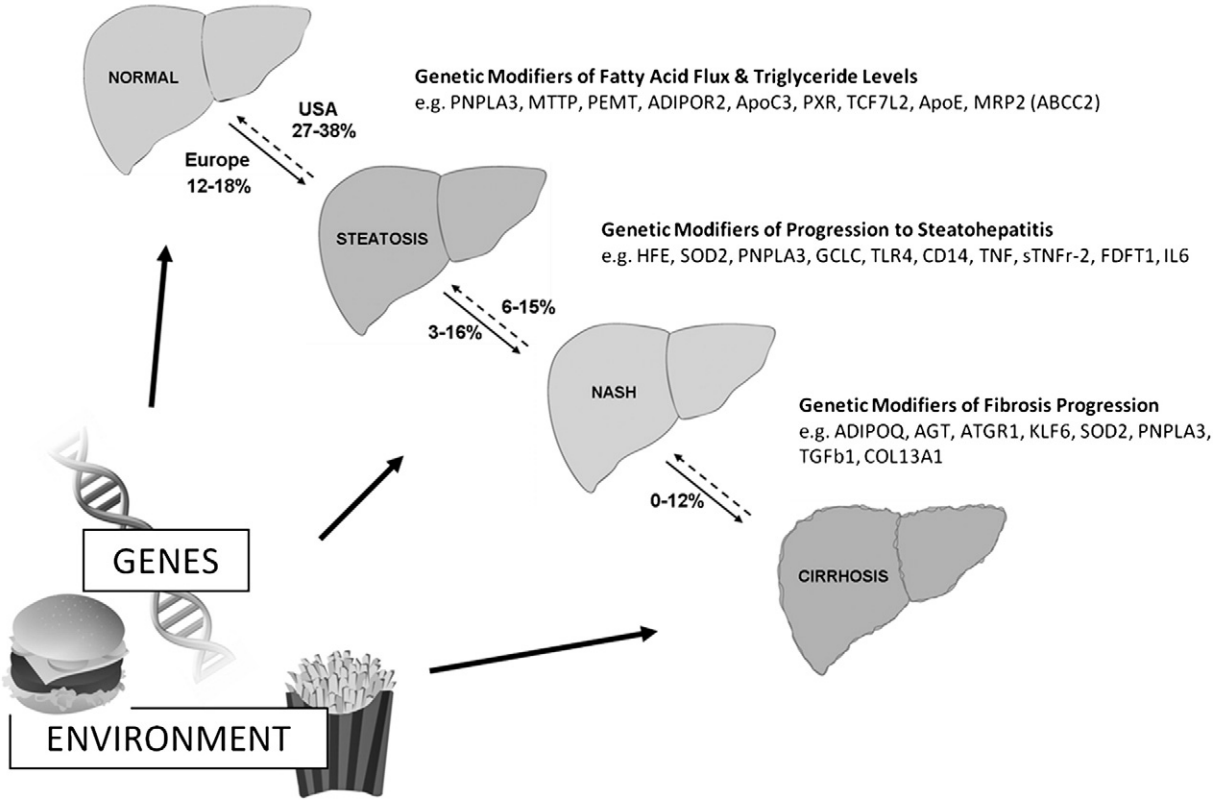

Fig. 1. Summary of genetic modifiers of progressive NAFLD.

persist for some time, these processes lead to stellate cell activation, collagen deposition and hepatic fibrosis [15]. In depth discussion of these mechanisms falls outside the scope of this review, however this framework will be adopted in our discussion of genetic modifiers.

3. Techniques for investigating the genetic basis of NAFLD/NASH

Until recently, the primary approach to the genetic study of complex disease traits was through case–control disease-association studies in man where candidate genes were selected on the basis of a putative role of their encoded proteins in disease pathogenesis. Being reliant on an *a priori* hypotheses for gene selection, these studies were limited to known candidates and therefore were not able to explore the potential role of other less obvious genes that may have an equal or greater influence on disease susceptibility.

In the ten years since the publication of the draft human genome there have been significant advances in our understanding of genomic variation. Several million single nucleotide polymorphisms (SNPs) have been described across individuals from diverse ethnic backgrounds [16–18]. This has paved the way for the development of SNP genotyping arrays and genome-wide association studies (GWAS) that have allowed the majority of common (minor allele frequency >5%) variability in the human genome to be simultaneously surveyed. The availability of high throughput next-generation sequencing offers the prospect of even more comprehensive characterisation of genomic variability, particularly the more rare genetic variants that are not effectively identified by GWAS but which may have a relatively large effect on disease risk. Greater discussion of these techniques falls outside the scope of this review but they are discussed more fully elsewhere [19–21].

The utility of the GWAS approach was demonstrated by one of the earliest reports describing simultaneous study of seven different complex diseases [22]. Since then, the number of GWAS studies in the literature has expanded exponentially. Amongst these, several liver-related diseases and traits have been studied. Factors that influence variation in biochemical liver function tests [23], drug induced liver injury [24,25], gallstone disease [26], primary biliary cirrhosis [27–29], NAFLD [30–32], hepatitis B persistence [33] and hepatitis C treatment response [34,35] have been identified. A welcome consequence of wider adoption of non-hypothesis driven GWAS techniques is that the loci identified are frequently novel and would not previously have been implicated in disease pathogenesis. However, as often neither biological function nor pathogenic mechanisms are known, such associations require further detailed study both to determine activity and to validate causality. In addition, candidate gene association studies have examined modifiers of disease progression and fibrosis [36,37].

4. Genome wide association studies in NAFLD/NASH research

To date, three GWAS scale studies have been reported in this field [30–32]. Each has captured new data and provided additional insights into the role of genomic variation in NASH pathogenesis (Table 1).

4.1. The first GWAS — Romeo et al.

The first NASH related GWAS was a genome-wide survey of non-synonymous sequence variation encompassing 9229 SNPs across a mixed population of Hispanic, African American and European ancestry derived from the Dallas Heart Study [30,38]. Although not based on direct assessment of steatosis in liver biopsy samples, the non-invasive proton magnetic resonance spectroscopy (^{1}H-MRS) technique used to assess hepatic steatosis is widely adopted in both human and murine studies [39,40]. The striking results of this study clearly identified the patatin-like phospholipase domain-containing 3