Nuclear Receptor Variants in Liver Disease

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Introduction to Nuclear Receptors

Liver metabolism is regulated by a network of specific transcription factors called nuclear receptors (NRs), which control many enzymes and transporters involved in steroidogenesis (NR class IA), reproduction and development (IB), circadian and basic metabolic functions (IC), bile acid and xenobiotic metabolism (IIA), and lipid metabolism and energy homeostasis (IIB and C) [1]. Gene variants of NRs have been identified and from a systematic point of view can be classified as diagnostic variants causing monogenic (‘mendelian’) liver diseases, predictive variants for hereditary liver diseases (in asymptomatic carriers) and variants resulting in a predisposition to polygenic (multifactorial/complex) liver diseases (genomic susceptibility variants in ‘at-risk’ individuals) [2]. In the past, gene variants for several monogenic hepatobiliary diseases have been identified, including progressive familial intrahepatic cholestasis types 1–3 (ATP8B1, ABCB11 and ABCB4 gene mutations, respectively), Dubin-Johnson syndrome (ABCC2), sitosterolemia (ABCG5/G8) and cystic fibrosis (ABCC7). In contrast, NR variants causing monogenic diseases appear to be scarce. They include familial partial lipodystrophy type 3 (PPARγ = NR1C3), maturity-onset diabetes of the young type 1 (HNF4α = NR2A1) and hemophilia type B due to mutated HNF4α and androgen receptor (AR = NR3C4) binding sites of the factor IX gene.

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NRs are regulatory molecules that orchestrate gene transcription in response to the presence or absence of specific ligands. They are characterized by a ligand-binding and a DNA-binding domain. NRs represent a central point of interaction between environment and gene regulation. They are the ‘hinge’ connecting endogenous and environmental stimuli, i.e. metabolites as ligands, with the cells’ transcriptional response. Of note, a schematic depiction of NR action in hepatocytes demonstrates a reduction in complexity and an increase in penetration of genetic variants from the sinusoids to the canaliculus [3].

Basics of Genomics

Fundamental technologies for genetic studies of NR variants include candidate variant detection (e.g. SNP arrays) for common pathogenic mutations and de novo sequencing by either conventional Sanger or next-generation methods. The earliest candidate gene studies focused on the nuclear peroxisome proliferator-activated receptor PPARγ (NR1C3). A single nucleotide polymorphism (SNP) resulting in the amino acid substitution p.P12A is known to be associated with noninsulin-dependent diabetes, BMI and dyslipidemia [4, 5]. NRs bind to a specific sequence in the promoter of target genes (hormone response elements) and activate transcription upon binding of the ligand. PPARγ binds as a heterodimer with the retinoid X receptor RXR (NR2B3) to a specific response element in target genes, with ligands comprising prostaglandin J2 and glitazones. The mutation p.P12A results in a defective receptor with respect to DNA binding and ligand-stimulated transactivation, and the association identified in candidate gene studies has been confirmed by genome-wide association studies (GWAS) and meta-analyses. The odds ratio (OR) conferred by the risk variant ranges from 1.14 to 1.25, but due to the high frequency of the risk allele (88%), the population-attributable risk for diabetes is approximately 25% [6]. In contrast, a recent meta-analysis indicated that the p.P12A polymorphism is not associated with risk for nonalcoholic fatty liver disease [7]. Stratified analysis using ethnicity indicated no significant association between the variant and the risk of nonalcoholic fatty liver disease in either Asian or Caucasian populations was detectable under any genetic model. These discrepancies might reflect complex gene-environment interactions in human populations, which can be dissected in experimental studies: using a p.P12A knock-in model, Auwerx and colleagues [8] demonstrated that mice bearing the AA genotype are leaner and have improved insulin sensitivity and plasma lipid profiles on chow diet. High-fat feeding eliminated the beneficial effects of the variant on adiposity, plasma lipids and insulin sensitivity. The underlying molecular mechanisms might involve changes in cofactor interaction and adiponectin signaling.

FXR Variants and Cholestasis

The central bile acid sensor FXR (farnesoid X receptor, NR1H4) is a critical regulator of hepatic bile acid metabolism [9]. A survey of genetic variation revealed an intriguing paucity of variants in the coding regions of NRs including FXR [10]. A comparison of variant frequencies in the sequences of NRs with drug metabolizing cytochrome P450 enzymes and ribosomal genes revealed less variation in coding areas, but a higher number of SNPs in the regulatory sequences of NRs [10]. Accordingly, an atlas of genetic influences on human blood metabolites based on GWAS with high-throughput metabolic profiling comprising 7,824 adult individuals from two European population studies reported genome-wide significant associations at 145 metabolic loci and their biochemical connectivity with more than 400 metabolites in human blood [11]. However, this included only one NR, the constitutive androstane receptor CAR (NR1I3).

The first study to associate genetic variation in FXR with a liver disease reported that a specific variant (p.M173T) confers susceptibility to intrahepatic cholestasis of pregnancy (OR = 3.2, p = 0.02) [12]. The coding regions and intron/exon boundaries of FXR were sequenced in 92 patients of mixed ethnicity; subsequently, a case-control study of allele frequencies of these variants in two independent cohorts was performed. Variants were cloned into an FXR expression plasmid and functional defects in either translation efficiency or activity, as well as repression of target genes (SHP1, OATP1B1) for the three FXR variants, c.-1g>t, p.M1V and p.M173T. The future challenge is to integrate the risks conferred by individual variants into ‘gene signatures’ based on multiple SNPs, as reported for combination of up to three risk variants in patients with intrahepatic cholestasis of pregnancy [13–15].

Mice lacking the bile acid sensor FXR are susceptible to gallstones when challenged with a high-fat high-cholesterol diet; furthermore, treatment with a synthetic FXR agonist increased biliary bile salt and phospholipid secretion, which restored cholesterol solubility and thereby
prevented gallstone formation [16]. Accordingly, we genotyped seven FXR SNPs in 1,004 individuals from Mexico, Chile and Germany [17]. After stratification for ethnicity and gender, the analysis of haplotypes (multiple linked SNPs) identified a common risk haplotype (c.-20647T, c.-1G, IVS7-31A) that was associated with gallstones in Mexican patients (OR = 2.1, p = 0.02). This finding was recapitulated in a recent study [18] based on sequencing the whole FXR gene in 30 gallstone patients. Association tests for 10 SNPs in 74 gallstone carriers and 133 controls resulted in weight- and gender-specific association with FXR variants (OR = 4.7, p = 0.02). The genetic association was supported by the effects of FXR variants on ileal FXR protein expression and target gene expression (ILBP, LRH-1 and FGF19).

From Pathway Analysis to Gene Networks

In contrast to a candidate study of a single gene, pathway analysis has the potential to identify novel associations. For example, bacterial translocation across the intestinal mucosa barrier represents a critical pathway leading to spontaneous bacterial peritonitis in patients with liver cirrhosis. We have shown that NOD2 gene variants (p.R702W, p.G908R, c.3020insC) known to affect mucosal barrier in Crohn’s disease occur significantly more often in patients with liver cirrhosis who develop spontaneous bacterial peritonitis (OR = 3.1, p = 0.001) [19]. Furthermore, additional variants in two other genes including FXR c.-1G>t were found to be associated with spontaneous bacterial peritonitis [20], resulting in a multiple gene model (table 1).

In fact, many more gene variants can be expected to regulate a liver phenotype, as highlighted by the identification of the macrophage-enriched metabolic network, which is associated with quantitative traits of the metabolic syndrome in both mice and humans, and consists of 1,406 genes [21, 22].

PXR Variants and Drug-Induced Liver Injury

Although functional pregnane X receptor (PXR = NR1I2) variants, in particular c.-25385T>C, have been associated with drug-induced liver injury (DILI) [23], GWAS indicate a predominant role of the HLA region in genetic susceptibility for DILI [24]. This finding points to the predominant relevance of individual immune-mediated pathomechanisms in the development of DILI. In contrast, GWAS have actually identified a role of other NR variants for liver-related traits, such as dyslipidemia. The screening of 19,840 individuals and a subsequent replication cohort of 20,623 individuals with 2.6 million SNPs resulted in 30 loci [25]. In this GWAS, serum LDL-cholesterol concentrations were associated with the common ABCG8 SNP p.D19H (effect size 15%), and the NR variant HNF4a p.T130I was shown to determine HDL-cholesterol levels (effect size 19%, risk allele frequency 3%).

The most powerful genetic study designs are longitudinal cohorts, which allow the evolution of early-life traits to be studied and can better define the genetic background and environmental modifiers as compared to cross-sectional studies. Individuals can be drawn from the full distribution of disease-associated traits, and rare variants with broader phenotypic effects can be identified [26]. The longitudinal cohort study in the Northern Finland Birth Cohort 1966 represents an example: overall, 329,091 SNPs were genotyped in 4,763 individuals, resulting in the identification of 21 loci [27]. An HDL-cholesterol locus was attributed to an LXRa (NR1H3) low-risk variant with a risk allele frequency of 42%; in contrast, it is remarkable that for LDL-cholesterol levels, an AR variant with an effect size of 30% could be identified in spite of its low-risk allele frequency (2%).

LRH-1 and Cancer

There is limited information on inherited risk factors modulating genetic susceptibility to cholangiocarcinoma (CCA), the second most common primary liver cancer with an unfavorable prognosis and poorly defined pathogenesis. A common variant in the NR5A2 (LRH-1, liver receptor homologue-1) gene has been identified as a genetic risk factor for pancreatic cancer in a recent GWAS [28]. Subsequent pathway analysis identified the strongest signal in the pancreatic development pathway (PDX1, NR5A2, HNF1α, HNF4γ = NR2A2) [29].

Table 1. Genetic risk factors for spontaneous bacterial peritonitis [20]

| Gene | SNP | OR   | 95% CI | p  |
|------|-----|------|--------|----|
| FXR  | c.-1G>T | 6.8   | 1.4–33 | 0.02 |
| NOD2 | p.R702W | 2.1   | 1.2–3.8 | 0.01 |
| TLR2 | c.-16934TT | 1.8   | 1.01–3.2 | 0.05 |

c = cDNA; NOD2 = nucleotide-binding oligomerization domain-containing protein; p = protein; TLR = Toll-like receptor.
Since NR5A2 is critically involved in embryonic development, bile salt metabolism and cholesterol transport, we assessed a potential contribution of the variant rs3790844 to genetic CCA risk modulation [Zimmer V, Lammert F; unpubl. observations]. In a large European-based CCA cohort, we genotyped this NR5A2 SNP in 226 CCA individuals and a total of 350 CCA-free controls. The rs3790844 [T] allele was overrepresented in the CCA group, providing a consistent association signal in allele- and genotype-based tests (OR = 1.3, p = 0.04). Of interest, the effect size appeared to be larger in the comparatively small intrahepatic CCA subgroup. These findings provide the first evidence for genetic risk modulation by the common NR5A2 variant, and suggest a novel role of this nuclear receptor in cholangiocarcinogenesis by functional mechanisms yet to be determined.

Key Messages

The examples of NR variants illustrate that transporter and few NR mutations may cause monogenic liver and metabolic diseases (e.g. HNF4α). Common NR variants modify complex liver and metabolic traits (e.g. FXR, NR5A2). Future risk assessment of hepatobiliary disease should include NR variant and/or expression ‘signatures’ or even ‘networks’ (e.g. intrahepatic cholestasis of pregnancy, gallstones).

Disclosure Statement

The authors declare no conflicts of interest related to this review.

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