Environmental exposure as a risk-modifying factor in liver diseases: Knowns and unknowns

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Abstract
Liver diseases are considered to predominantly possess an inherited or xenobiotic etiology. However, inheritance drives the ability to appropriately adapt to environmental stressors, and disease is the culmination of a maladaptive response. Thus “pure” genetic and “pure” xenobiotic liver diseases are modified by each other and other factors, identified or unknown. The purpose of this review is to highlight the knowledgebase of environmental exposure as a potential risk modifying agent for the development of liver disease by other causes. This exercise is not to argue that all liver diseases have an environmental component, but to challenge the assumption that the current state of our knowledge is sufficient in all cases to conclusively dismiss this as a possibility. This review also discusses key new tools and approaches that will likely be critical to address this question in the future. Taken together, identifying the key gaps in our understanding is critical for the field to move forward, or at the very least to “know what we don’t know.”

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1. Introduction

...informed ignorance provides the natural state of mind for research scientists at the ever-shifting frontiers of knowledge. People who believe themselves ignorant of nothing have neither looked for, nor stumbled upon, the boundary between what is known and unknown in the cosmos.

This famous quote by the astrophysicist Neil deGrasse Tyson highlights the difficulty in making conclusions on what is known when all data are not available. This review will highlight the knowledgebase of environmental exposure as a potential risk-modifying agent for the development of liver disease by other causes (Table 1). We will focus on what is known, as well as prospects for better understanding (i.e., unknowns).

1.1. The liver as a target organ of toxicity

The liver plays multiple roles in the intact organism. It is critical for maintaining systemic metabolic homeostasis and for synthesis of lipids and carbohydrates. The liver is also the site of synthesis of the majority of plasma proteins (e.g., albumin and clotting factors), and it synthesizes and excretes bile acids, which are critical for normal uptake of vitamins and lipids, as well as for and excretion of xenobiotics. The liver also has a unique immune function, as it serves as a key regulator of tolerance, immunity and crosstalk between the innate and adaptive immune responses.

The liver is a key organ for xenobiotic metabolism. The strategic location of the liver between the intestinal tract and the rest of the body makes it a critical physical and biochemical barrier between absorbed xenobiotics and the systemic circulation. It is therefore not surprising that the liver has a very high capacity for phase I and II metabolic processes, which is predominantly responsible for the well-known ‘1st pass effect’ in xenobiotic metabolism. As the main detoxifying organ in the body, the liver has a high likelihood of toxic injury. Indeed, liver injury is a common reason for cessation of preclinical screening of new drugs, as well as removal of approved drugs from the market. Moreover, ~30% of common workplace chemicals are associated with hepatotoxicity.

The death of injured hepatic cells may be viewed an adaptive response that prevents the accumulation of irreversibly-damaged cells in the organ; as such, this process may protect against organ aging and cancer. Indeed, the high potential of hepatic injury by xenobiotics is offset by the tremendous regenerative capacity of the organ. This capacity distinguishes the liver from other encapsulated organs (e.g., the brain, heart, and lungs) that are far less able to replace functional tissue once it has been destroyed. In experimental animals, the liver can fully regenerate after surgical removal of 2/3 of the organ within 7–10 days. Although hepatocytes rarely proliferate in the healthy adult liver, virtually all surviving hepatocytes replicate at least once after partial hepatectomy or acute toxic liver injury. Residual hepatocytes upregulate both proliferative and liver-specific gene expression to preserve tissue specific function. During liver regeneration, a complex network of cytokines, growth factors, kinases, and transcription factors drive hepatocytes out of the G0 phase to enter and progress through replication.

In addition to hepatocyte proliferation, there is a tightly coordinated response to complement the regenerative process (e.g., angiogenesis, extracellular matrix metabolism).

The complex and synchronized regenerative response in liver can be perturbed and thereby can impact normal tissue recovery from injury or damage. Indeed, it is now clear that impaired or altered regeneration and/or restitution is critical to the chronicity of numerous hepatic diseases. Chronic liver injury, coupled with impaired/incomplete regeneration and restitution of said injury, leads to an almost universal endpoint of collagenous scarring of the liver (i.e., fibrosis), regardless of etiology. It is hypothesized that hepatic fibrosis is initiated predominantly by a failure of the liver to sufficiently regenerate itself after chronic injury. Given the clinical implications of fibrosis/cirrhosis, and the relative difficulty of reversing this pathology, research in liver disease has focused on better means to identify and prevent fibrosis progression.

### Table 1 Factors that influence the development and severity of liver diseases.

| Liver disease                  | Genetics | Lifestyle/comorbidities | Environmental toxicants | Microbiome |
|-------------------------------|----------|-------------------------|-------------------------|------------|
| Inherited liver diseases      | ++       | +                       | –                       | +          |
| Biliary atresia               | ++       | + (+)                   | + (+)                   | +          |
| Primary biliary cholangitis   | +        | –                       | + (+)                   | +          |
| Primary sclerosing cholangitis| ++       | –                       | –                       | +          |
| Autoimmune hepatitis          | ++       | + 21–24                 | + 23–25                 | +          |
| Direct DILI                    | ++       | + 30–32                 | + 33–35                 | +          |
| Idiosyncratic DILI             | +        | + 36                    | –                       | –          |
| Viral hepatitis                | ++       | + 11–42                 | + (+) 34–44             | –          |
| NAFLD/MAFLD                   | ++       | + 45                    | + (+) 46–49             | + (+) 50,51|
| ALD                            | +        | + 52                    | –                       | + (+) 51,53|
| TAFLD/TASH                    | –        | –                       | + (+) 46,54             | +          |

–, no known association or not classifiable. Evidence inadequate in humans and limited in experimental models.
+., possible association. Limited evidence in humans and in experimental models.
++, probable association. Limited evidence in humans and sufficient in experimental models.
++++, known association and/or primary cause. Clear cause and effect association is known.
*Sexual dimorphism, familial associations, linkage disequilibrium, etc.
†Viral infections, metabolic syndrome, alcohol, underlying liver disease, etc.
*Exposure to environmental/occupational chemicals or toxins, natural or anthropogenic, that was not self-administered.
*Altered microbiome/dysbiosis in diseased patients or in animal models.
1.2. Liver disease at the crossroads of environment and genetics

The broader definition of environmental exposure includes any non-inherited influences. For the context of this review, we will define environmental exposure more narrowly to exposure to environmental chemicals or toxins, natural or anthropogenic, that was not self-administered (e.g., alcohol) or the result of a pathogen infection (e.g., hepatitis viruses). Although they are not synonymous, we will also not distinguish occupational from true environmental exposures.

Liver diseases are considered to predominantly possess an inherited (e.g., Wilson’s disease) or xenobiotic (e.g., alcohol-related liver disease; ALD) etiology. However, the risk of both genetic- and xenobiotic-derived liver diseases are modified by other factors (see Fig. 1). For example, the penetrance of Wilson’s disease is lower than expectations based on allele frequency, and it can be prevented by diet modification and/or pharmacotherapy. Likewise, only a fraction of even the heaviest drinkers develops clinically relevant ALD. Judith Stern is quoted as stating, “Genetics loads the gun, but the environment pulls the trigger.” This statement aptly emphasizes the point that inheritance drives the ability to appropriately adapt to environmental stressors, and that disease is the culmination of a maladaptive response. This is certainly true for liver diseases, in which even “pure” genetic and “pure” xenobiotic diseases are modified by each other and other factors, known or unknown (Fig. 1).

2. Inherited liver diseases and environmental exposure

Congenital variations in genes critical to metabolic pathways can lead to deficiencies and/or accumulation of toxic intermediates that damage target organs. As mentioned above, the liver plays a key role in maintaining overall metabolic function of the organism. As such, inherited variations in metabolic pathways often manifest in hepatic or liver-dependent extra-hepatic phenotypes. These can often be severe and subsequently present very early in the lifespan. Indeed, inborn errors of metabolism are a common reason for pediatric liver transplantation.

In general, the impact of environmental exposure on the penetrance and severity of inherited liver diseases is not well-known. These liver diseases are very rare and possess a clear, and often known, genetic component. Nevertheless, a role of environmental exposure in modifying risk cannot be ruled out in all cases. For example, Alagille syndrome is an inherited disease that causes bile duct paucity and cholestasis (see Section 3, below). Key autosomal dominant variants in the Notch signaling pathway have been established as causal for the cholestatic liver disease associated with Alagille syndrome. However, despite the autosomal dominance of the disease, penetrance and severity has been variable, even within the same family. Some case reports of discordant disease and clinical phenotypes in monozygotic twins with Alagille syndrome has led to the speculation that environmental factors may contribute to disease severity.

Some inherited liver diseases are well-known to have environmentally modifiable responses, at least in other target organs. For example, α1-antitrypsin (AAT) deficiency is caused by an inherited deficit in the generation of AAT by the liver. Although liver injury often presents in AAT deficiency, the most common complication is lung disease, where AAT is required to prevent localized damage by neutrophil elastase. Lung disease associated with AAT-deficiency is modifiable by environmental exposures. Less is known about the interaction between AAT-deficiency and environmental exposure and hepatic disease. However, recent studies suggest that this deficiency may be also an independent risk factor for cirrhosis by other causes, which suggests a genetics/environment interaction is at least possible. Likewise, lung complications associated with polymorphisms in the cystic fibrosis transmembrane receptor (CFTR) pathway are known to be heavily influenced by environmental exposures. Although less is known about hepatic complications of CFTR polymorphisms, some environmental chemicals that bioaccumulate in the liver are known to interact with the CFTR pathway (e.g., fungicides and perfluoralkyl compounds), which could exacerbate inherited CFTR dysfunction in the liver. Recent studies have indicated that several environmental chemicals may alter metal metabolism and transport within the cell, including dioxins and poly-chlorinated biphenyls. Environmental exposures that alter metal transport/metabolism could also potentially impact inherited diseases of metal dyshomeostasis (e.g., Wilson’s disease, and hereditary hemochromatosis), although this needs to pursued experimentally. In general, however, the potential for such interactions between inherited diseases and environmental exposures has not been extensively addressed and mechanistic insight is subsequently lacking.

3. Cholestatic liver diseases

Cholestasis refers to disruption of proper bile acid and bilirubin metabolism, resulting in their increased levels in blood and peripheral tissues. Cholestasis generally falls into two categories: intrahepatic and extrahepatic (obstructive) cholestasis.

Figure 1  The totality of risk of liver diseases. Liver diseases have primary underlying causes, be they genetic (e.g., inborn errors of metabolism), lifestyle (e.g., metabolic syndrome, alcohol consumption), viral infection (e.g., HBV/HCV infection) and drugs (e.g., acetaminophen overdose). However, the interindividual risk for the development of liver disease is also impacted by other factors that influence the severity and outcome driven by the primary cause(s). Comorbidities influence by lifestyle, coinfections or other underlying diseases, and drug exposure may all positively and negative influence disease severity and progression. Moreover, risk modifiers, such as genetic susceptibility, alterations in the microbiome, as well as biologic (e.g., age) and sociodemographic health disparities influence interindividual susceptibility. Lastly, the totality of exposure to environmental chemicals (i.e., the “exposome”) may influence disease progression.
Intrahepatic cholestasis can be primary or secondary to other liver diseases (e.g., drug-induced liver injury; DILI, and ALD). These diseases can also be developmental or acquired.

### 3.1. Biliary atresia

Biliary atresia (BA) is a developmental disorder that impacts the extrahepatic bile ducts of neonates. It is the most common cause of pediatric liver transplantation, as the progressive damage and fibrosis of the bile ducts lead to complete obstruction. The underlying etiology is incompletely understood but appears to involve dyshomeostasis between the innate and adaptive immune responses, leading to destruction of the bile duct epithelial cells. In contrast to inherited diseases of extrahepatic cholestasis (e.g., Alagille syndrome, see above), there is not a clear single genetic susceptibility factor driving BA. There are several polymorphisms that are associated with BA susceptibility, but the allele frequency and penetrance cannot explain the entirety of the disease.

BA is well-known to have regional variation in the disease incidence worldwide. This and other factors led to speculation that BA and other pediatric cholangiopathies may have a xenobiotic component. There are data supporting the hypothesis that in utero exposure to viral infections (e.g., rotavirus) may be a triggering factor in BA. Building on observations of naturally-occurring outbreaks of BA in Australian livestock, researchers isolated a plant toxin that was demonstrated to cause experimental BA in zebrafish, mice and in human biliary epithelial cells. Although it is unlikely that humans will be exposed to this plant toxin and local outbreaks of human BA have not been identified, these results support the notion that environmental exposure can cause BA. To date, no anthropogenic product has been linked to BA.

### 3.2. Primary biliary cholangitis

Primary biliary cholangitis (PBC) is a rare chronic autoimmune disease in which the biliary epithelial cells of the small to medium intrahepatic bile ducts are progressively destroyed. PBC can manifest at any time in the lifespan but is generally an adult-onset disease. PBC is also sexually dimorphic, with a much higher prevalence in women. Although PBC can respond to interventional strategies (e.g., immune suppression, disease modifying agents and antifibrotics), it often progresses to biliary cirrhosis.

Although the initiation of PBC is incompletely understood, it is thought that the breaking of self-tolerance to the biliary epithelium is the key step; the presence of antimitochondrial antibodies is a specific and biliary epithelium autoantibodies. The incidence of PBC has long been thought to have an environmental trigger or contributing to AIH. Indeed, DILI (see below) often has components of AIH. Beyond viral and specific drug-induced cases of AIH, there have been few examples of environmental exposure potentially causing or contributing to AIH. There is a relatively strong link to the development of AIH and other autoimmune diseases to trichloroethylene exposure. Based on studies in animal models, it is hypothesized that trichloroethylene may trigger AIH by preventing activation-induced apoptosis of CD4+ T cells, while simultaneously...
creating protein adducts that can serve as neoantigens for this expanded population of activated T cells\textsuperscript{77}. Whether this mechanism is unifying for all environmental exposures that may contribute to AIH is unclear.

5. Drug-induced liver injury

DILI is defined as an acute hepatotoxic event that can be directly linked to the ingestion of drugs. DILI is the most common cause of acute liver failure in middle- and high-income countries and a common cause for liver transplantation\textsuperscript{102}. The diagnosis of DILI relies not only on the exclusion of other causes, but also on timing of injury after drug administration has been started, recovery after the drug has been discontinued, and relapse of injury after reintroduction of the drug\textsuperscript{103}. DILI is further categorized into two main groups, direct and idiosyncratic\textsuperscript{103}.

5.1. Direct DILI (dDILI)

dDILI is characterized by predictable, dose-dependent liver injury by compounds known to be intrinsically hepatotoxic in humans and in preclinical models\textsuperscript{103}. The pattern of injury is usually specific to the compound ingested\textsuperscript{103}, but is most frequently characterized by a rapid onset and acute hepatic necrosis coupled with elevations of serum liver enzymes (e.g., AST and ALT). Less frequently, dDILI may show a cholestatic or mixed phenotype. The most common and well-characterized example of dDILI is acetaminophen (APAP) toxicity\textsuperscript{104}, but is also common for other classes of drugs (e.g., chemotherapeutics)\textsuperscript{103}.

The dose-dependence of dDILI implies that it is commonly the result of accidental or intentional overdose\textsuperscript{102}. However, there are nevertheless cases in which patients develop dDILI within the normal therapeutic range of the compound. These factors have fueled discussion as to whether other factors (genetic or lifestyle) modify interindividual risk for the development of dDILI\textsuperscript{105}. There are a few examples in which genetic predispositions for dDILI have been identified, most of which are centered on polymorphisms that are few examples in which genetic predispositions for dDILI have been identified, it is generally assumed that genetic variance alone does not completely explain interindividual risk of dDILI development. Indeed, several host-factors have been suggested to increase the risk of dDILI, including sex, age, lifestyle (e.g., alcohol consumption, nutrition), co-medications and underlying diseases that may drive differential pharmacokinetic/pharmacodynamic responses to drugs\textsuperscript{18}. However, many of these linkages also have contradictory findings in the literature (e.g., Ref.\textsuperscript{107}). There are few linkages between environmental exposure and the risk of dDILI, although several environmental compounds can contribute to altered pharmacokinetic/pharmacodynamic responses in hosts\textsuperscript{108,109}. For example, several environmental chemicals have been demonstrated to interact with drug transporters\textsuperscript{110}. Given the critical importance that altered drug metabolism and transport is hypothesized to play in dDILI\textsuperscript{111,112}, such an interaction could lead to intoxication. This area should be explored in future research.

6. Viral hepatitis

Several viruses are hepatotrophic and can cause liver injury. Most hepatitis viruses only cause acute infections that are usually subclinical and self-limiting in immunocompetent adults (e.g., hepatitis A and E)\textsuperscript{113,114}. In contrast, hepatitis B virus (HBV) and HCV often transition to a chronic infection and liver disease. The natural history of liver disease from chronic HBV/HCV infection is similar to fatty liver disease (see Section 7). Despite the existence of an effective vaccine (HBV) and antiviral therapies (HBV and HCV), chronic viral liver disease remains a leading cause of cirrhosis, hepatocellular carcinoma (HCC), liver transplantation and death worldwide\textsuperscript{115}. There are genetic factors that influence host susceptibility to HBV and HCV infection and liver injury\textsuperscript{39,40}. There are also some data indicating an interaction between other factors and viral pathogenesis, the most notable is coexposure to aflatoxin, a regional food-born contaminant (see Section 8)\textsuperscript{41,42}. For example, alcohol consumption is a known risk factor severe liver disease during HBV/HCV infection\textsuperscript{41,42}. However, beyond these findings, few interactions between environmental exposure and viral liver disease have been identified.

7. Fatty liver diseases (ALD, NAFLD/MAFLD, TAFLD)

Chronic fatty liver diseases are a spectrum of diseases ranging from simple steatosis, to active inflammation (steatohepatitis) to fibrosis/cirrhosis, and potentially HCC (see Section 8, below). The principle primary causes of fatty liver diseases are alcohol...
consumption (i.e., ALD) and obesity/metabolic syndrome, which was originally coined nonalcoholic fatty liver disease (NAFLD). The natural history and pathologic progression of both diseases are similar, and the underlying causes are usually only determined by medical history questionnaires. Although there are clear cases of “pure” ALD (i.e., liver disease in lean heavy drinkers) and NAFLD (i.e., liver disease in obese non-drinkers), it is also clear that risk factors and lifestyles often overlap. Moreover, as reviewed elsewhere in this issue (Schnegelberger et al.), there are now data indicating that environmental toxicant exposure can lead to fatty liver disease. This understanding has led to the recent reframing of fatty liver diseases under the inclusive umbrella term of metabolic-associated fatty liver disease (MAFLD).

It is estimated that up to 1/3 of the global population has fatty liver disease and it is considered to be a looming health burden. Regardless of primary causes, the risk of developing severe fatty liver disease is well-known to be modified by other factors both genetic and lifestyle. In contrast to the other liver diseases discussed in this review, there are several lines of evidence that indicate that environmental toxicants may be a risk factor for the development and progression of fatty liver diseases, especially due to obesity/insulin resistance. There are relatively strong data linking exposure to persistent organic pollutants (e.g., dioxin), volatile organic compounds (e.g., vinyl chloride; VC), metals (e.g., arsenic) as well as some herbicides/fungicides with the development of experimental and human fatty liver disease. Although the underlying mechanisms of potential interactions between environmental exposure and fatty liver diseases are still being elucidated, the environmental agents can generally be classed as endocrine-, metabolism- and signaling-disrupting chemicals that potentially exacerbate the metabolic dysfunction and/or disease progression in fatty liver diseases.

Environmental exposure in some cases has been identified as a primary cause in the development of fatty liver diseases, coined toxicant-associated fatty liver disease (TAFID). It is likely that one of the clearest examples of an environmental liver disease is VC exposure. VC is chiefly employed in the rubber manufacturing industry as it is an intermediary monomer used in the production of the polymer polyvinyl chloride. One of the first cases to report hepatic injury caused by VC exposure dated back to 1974 when 3 polyvinyl chloride factory workers were diagnosed with hepatic hemangiosarcoma, a rare form of liver cancer. More recently, the prevalence of TAFID was identified in VC workers. TAFID is a progressive form of fatty liver disease that bears some resemblance to NAFLD. While its histological characteristics are similar to ALD/NAFLD, TAFID has been associated with normal liver enzyme levels, making it more challenging to diagnose. It is nevertheless clear that at high enough exposure levels, VC is sufficient to cause significant liver disease. At least in animal models, exposure to several environmental chemicals is sufficient to also cause fatty liver disease.

8. Environmental exposure and primary liver cancers

Primary liver cancer, predominantly HCC, is the 6th most common cancer worldwide. Treatment options for non-resectable HCC and other liver cancers are limited and the 5-year survival rate for HCC remains and is the 2nd most lethal solid cancer. In regions in which HBV infection and exposure to aflatoxin is not endemic, HCC occurs almost exclusively on the background on severe cirrhosis, which is the primary risk factor for HCC development. The incidence of HCC has been increasing in recent years, which may be a reflection of an increase in underlying liver disease in the population (e.g., MAFLD; see Section 7). Moreover, as the risk of HCC increases with the length of cirrhosis, improvements in the clinical management of compensated (i.e., “stable”) cirrhosis may also contribute to this increasing incidence of HCC.

As HCC and other liver cancers are generally dependent upon preexisting chronic liver disease, environmental exposures discussed in previous sections that contribute to that liver disease by extension may contribute to the risk of HCC. For example, exposure to the mutagen aflatoxin greatly increases the risk of the development of HCC by HBV (and possibly HCV) infection. Moreover, exposure to genotoxic chemicals may be an independent risk factor for HCC occurrence. Exposure to a myriad of such chemicals has been linked with at least an increase in HCC incidence, including polyhalogenated biphenyls, volatile organic compounds (e.g., VC benzo[a]pyrene and arsenic). It is hypothesized that clonal expansion of cells mutated by these agents during cirrhosis accelerates the risk of progression to HCC.

9. Altered liver regeneration as a unifying mechanism?

As mentioned in Section 1.1, the ability of the liver to regenerate after injury affords a unique protective mechanism for this organ against damage. The development of chronic liver diseases is almost universally present on the background of genetic/acquired impairment of regeneration and restitution from injury, which allows damage to accumulate. Even acute liver injury, such as dILI caused by APAP overdose (see Section 5.1) is driven, at least in part, by the rate of hepatic regeneration after injury. Thus, impaired regeneration may be a unifying mechanism that influences liver injury and liver disease, regardless of underlying causes.

As described in Section 1.1, the regenerative response is a complex and tightly coordinated process that by extension is sensitive to perturbation. Some environmental chemicals have been described to impair liver regeneration, at least in experimental models, including heavy metals and dioxin-like chemicals, but information on other chemicals is limited. Conversely, some compounds (e.g., organochlorine pesticides, phthalates and perfluoroalkyl chemicals) have been demonstrated to enhance liver proliferation and cause hepatomegaly. The interplay between altered proliferation has implications not only for liver disease, but also for hepatocarcinogenesis (see Section 8, above).

10. Environmental exposure and liver disease: Prospects 'omics' and big data

The lack of information regarding a potential role of environmental exposure in some liver diseases is understandable. Some of these diseases (e.g., developmental liver diseases) are extremely rare and the prevalence even in large institutional catchments may be too low for any reasonably-powered assessment of risk-modifying agents. This limitation has been partially addressed by collaborative cohorting and biobanking across institutions; these cohorts exist for several liver diseases, including DILI, developmental/pediatric liver diseases, NAFLD and others. These advances have led to other issues, namely that it is difficult to manage and analyze large, heterogeneous multiplatform datasets to achieve meaningful results. These issues have lessened with continued development of machine learning toagnostically
analyze ‘Big Data’ and metadata\textsuperscript{152,153}. These approaches offer the opportunity to generate new hypotheses that explain interindividual risk of disease development (\textit{i.e.}, precision medicine). Some promising platforms relevant to questions regarding the role of environmental exposure in liver diseases are discussed below.

### 10.1. Microbiome

The microbiome, especially the gut microbiome, is now established as a dynamic and diverse compartment that plays key roles in mediating health and disease. Given the proximal location of the liver immediately downstream of the gastrointestinal tract, it is not surprising that the role of the gut microbiome in liver disease has been extensively studied\textsuperscript{156}. It can be argued that our understanding of the bidirectional ‘gut–liver axis’ has coevolved with our understanding of liver disease for at least the last half-century\textsuperscript{155}. As summarized in Table 1, almost every liver disease covered in this review has been associated with an altered gut microbiome\textsuperscript{156}. These associations have the exciting potential for the development of non-invasive biomarkers of disease phenotype\textsuperscript{50}. Whether these changes are causally involved in disease development also has larger implications for precision medicine and targeted interventive strategies\textsuperscript{50}. The latter point is incompletely understood in the context of most liver diseases, with some exciting exceptions. For example, recent work indicates that bacteriophage delivery to selectively target specific bacterial species is protective in experimental and human ALD\textsuperscript{53}. Another area of microbiome research that has been heavily studied is the influence of environmental chemicals on gut microbiome alpha- and beta-diversity, given the high level of potential exposure of this compartment\textsuperscript{157–159}. Integrative incorporation of these knowledgebases could yield new information and insight into the potential interaction between environmental exposure and liver diseases.

### 10.2. Geospatial studies

Health disparities are a complicated subject, driven by potential or established differences in genetics, culture and socioeconomic status\textsuperscript{160}. Liver diseases, like most diseases, are well-known to show categorical health disparities within a given population\textsuperscript{160–162}. It is also understood that liver diseases often show inter- and intra-regional variability in the incidence, severity, and outcomes\textsuperscript{162,163}. More recently, micro-regional (\textit{e.g.}, county- or even neighborhood-level) analysis of liver disease mortality has indicated that there are ‘hot-spots’ of liver disease\textsuperscript{164,165}. This level of geospatial analysis takes better into account local variation in sociodemographics, risk factors and access-to-care. One of the components of consideration in local health disparities is differential exposure to environmental chemicals that may impact health\textsuperscript{166}. It may not be surprising that local hotspots of environmental exposure often overlap with racial and socioeconomic disparity\textsuperscript{166}. As mentioned above, some geospatial analyses have linked specific liver diseases to local environmental exposures (\textit{e.g.}, Refs\textsuperscript{11–13}), but this is an area that should be further developed.

### 10.3. Exposomics

As mentioned above, disparities in exposure to environmental hazards often overlaps with other sociodemographic disparities\textsuperscript{167}. As such, geospatial analysis has limited value to inform on underlying causes without being integrated with other analyses (\textit{e.g.}, exposure assessment). Moreover, single exposure analysis does not factor in the dynamics of mixtures in environmental exposure and by design is restricted to hypothesis-driven approaches\textsuperscript{168}. These limitations have led to the development of the concept of the “exposome”, in which the totality of exposure would be measured, analogous to other ‘omic’ approaches\textsuperscript{169,170}. This field is rapidly developing in conjunction with machine learning. There is high potential for exposomics to integrate with other ‘omics’ (\textit{e.g.}, genomics, transcriptomics, etc.) in a multiomic platform to yield new information in disease research\textsuperscript{171–173}, including liver diseases\textsuperscript{74}.

### 11. Summary and conclusions. Do we know what we don’t know?

Exposure to environmental chemicals is ubiquitous and even neonates have detectable levels of anthropogenic environmental chemicals in their blood and this exposure may be a key factor in the developmental origins of disease\textsuperscript{175,176}. Importantly, several of these chemicals are known hepatotoxins (\textit{e.g.}, heavy metals)\textsuperscript{177}. As outlined above (see Table 1), there are some examples in which the weight-of-evidence strongly supports at least a modifying role of environmental exposure and the development and severity of liver diseases (\textit{e.g.}, PBC and NAFLD/MAFLD).

As a field, it is critical to direct research to address the “unknowns”, \textit{i.e.}, liver diseases for which little is known regarding the impact of environmental exposure (Table 1). These would include inherited liver diseases, PSC, iDILI and ALD (Table 1). This gap in our understanding is especially apparent for liver diseases in which the natural history is similar, but there is differential understanding of this potential interaction. For example, few studies have investigated the possible influence of environmental exposure in ALD, although it is acknowledged that the pathogenesis of ALD shares many similarities to NAFLD/MAFLD, where this issue has been studied extensively\textsuperscript{46–49}. Likewise, although much is known about PBC and environmental exposure, there is little information for PSC, which shares mechanisms of disease of at least initiation (\textit{e.g.}, breakage of self-tolerance). These gaps should be filled.

The purpose of this review is not to argue that all liver diseases have an environmental component, but to challenge the assumption that the current state of our knowledge is sufficient in all cases to conclusively dismiss this as a possibility. This review identified key new tools and approaches that will likely be critical in addressing these questions in the future. Taken together, identifying the key gaps in our understanding is critical for the field to move forward, or at the very least to “know what we don’t know.”

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### Author contributions

Juliane I Beier: drafting of the manuscript, and critical revision. Gavin E. Arteel: drafting of the manuscript, and critical revision. All authors: final approval of manuscript.

### Conflicts of interest

The authors declare no conflicts of interest.
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