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Studying the Exposome to Understand the Environmental Determinants of Complex Liver Diseases

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The term “exposome” was coined in 2005 by Dr. Christopher Wild to highlight the need to develop a complement to the genome that considers the role of environment in disease risk. Originally, the concept of the exposome was used to denote the accumulated lifetime environmental exposures encountered by an individual beginning *in utero*.⁽¹⁾ In 2014, this definition was redefined to include the cumulative effect of environmental exposures and biological responses, including the physio- and psychosocial milieu.^(2,3) Importantly, this definition recognizes that not all individual exposures need to be measured to characterize the exposome, but rather their cumulative effect. Exogenous chemicals can be acquired from multiple exposure routes, such as air, water, surfaces, and dietary/lifestyle choices, throughout the life course. Internal sources of exposure, such as inflammation, oxidative stress, and aging, also exist (Fig. 1).

Defining the Exposome

Critically, the extended concept of the exposome distances it from traditional toxicology and epidemiology, which is largely focused on studying well-defined hypotheses testing single chemical effects. Although these studies provide important insights into recognized environmental hazards and underlying toxic mechanisms, there are limited examples of complex diseases occurring as a result of a single exposure (just as complex diseases are not a consequence of a single genetic disruption).

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Measuring the Exposome

The two main strategies for exposome research can be defined as “bottom-up” or “top-down” approaches.⁽⁴⁾ (Fig. 2A,B) The bottom-up approach focuses first on characterizing external exposures in at-risk cohorts hypothesized to be associated with disease, often using direct methods, such as personal exposure monitoring through various technologies (e.g., sensors, mobile phones), to quantitate exposure levels like air pollution or activity level. Exposures associated with future disease development can be followed up by comprehensive interrogation of the internal exposome to identify associated differences in pathway and network analysis. By contrast, the top-down approach focuses on untargeted approaches using biological samples from cases and controls in order to systematically assess the relationship between the internal dose of an exposure and disease risk using an exposome-wide association study framework. Top associations between disease (or outcomes) and exposures (or biological responses) are then used to identify possible external exposures (i.e., parent compounds) driving disease risk and develop new hypotheses to study mechanisms underlying environment-disease relationships.

Regardless of the approach, measuring the exposome attempts to capture components of the “toxicological paradigm,” which outlines the different biological phases of an exposure. Figure 3 outlines the components of the toxicological process (thin solid lines) and associated susceptibility factors (dotted lines), the latter of which can be altered by genetics, age, and/or sex and other risk factors such as nutritional status (including obesity and malnutrition) and presence of concomitant disease. These susceptibility factors determine whether and when subjects are exposed to a biologically effective dose of environmental toxicants. At these doses, processes such as DNA methylation, gene expression, and microbiome homeostasis are disrupted, generating preclinical effects, which eventually lead to liver disease.

Evolving Methods in the Study of the Exposome

TECHNOLOGICAL ADVANCES IN MASS SPECTROMETRY

In order to characterize the role of the exposome in liver disease pathogenesis, it is critical to develop technologies that can measure environmental exposures at a resolution analogous to that available for genotyping. Conceptually, this requires measuring external and internal exposures as well as capturing biological alterations important for health. To date, metabolomics approaches based on high-resolution mass spectrometry (HRMS) have been identified as a key technology for profiling the human exposome.⁽⁵⁾ Although biological effects of exposures can be quantified using a variety of large-scale “-omics” platforms (Table 1), only metabolomics provides a unified assessment of internal exposure and biological effect by being able to detect exogenous chemicals and alterations in metabolic pathways.⁽⁶⁾ Indeed, the number of detectable features in human plasma using metabolomics has grown exponentially, from a few dozen to up to 8 million.⁽⁵⁾ One of the key technologies is ultra-HRMS (UHRMS), which can detect more than 1 million chemical signals in the blood, even at concentrations up to 1,000 times lower than endogenous signals.⁽⁷⁾ When combined with liquid chromatography or gas chromatography, UHRMS can

comprehensively characterize both exo- and endogenous molecules that comprise the exposome.⁽⁵⁾

STATISTICAL APPROACHES TO EXPOSOME-WIDE ASSOCIATION STUDIES AND MULTIOMIC INTEGRATION

To date, there is no single statistical standard for the analysis of exposome-wide associated studies (EWAS). Previous studies have focused only on single exposures or a handful of exposures, rather than a broad range of both external and internal features. Bioinformatics approaches in EWAS will be largely dependent on the hypothesis in question, availability of cross-sectional and longitudinal data, and methods used to capture the exposome. Two of the largest proposed EWAS studies, HELIX (Human Early Life Exposome) and EXPOsOMICS, propose different statistical methods, but their overall approach is similar.^(8,9) The first phase of data analytics involves *variable selection*, or the identification of biologically significant exposomic features that are statistically associated with outcomes of interest after correction for multiple testing. The second phase involves *biological association*, linking exposomic features with biological pathways using pathway analysis, networks, and clustering techniques. The third phase involves generating *risk estimates* from multiple exposures for the purposes of treatment and public health policy. When utilizing high-resolution metabolomics to measure the exposome, data analytics for high-throughput studies in metabolomics and genomics can be leveraged for EWAS. A nonexhaustive list of statistical techniques and resources that have previously been used in environmental surveys or proposed EWAS can be found in Table 2.

One of the challenges in exposome research lies in understanding the relationships between exposures and biological response profiles that could be drivers of disease processes. A wide-reaching, multidimensional, and integrated omics approach incorporating systems biology provides one of the best opportunities for novel discovery and hypothesis generation of the molecular basis of disease.⁽¹⁰⁾ Arguably, these discoveries cannot be accomplished without including genome discovery. By combining high-throughput multiomics evaluation in case-control studies, candidate agents associated with disease can be identified and, ideally, tested using *in vivo* and *in vitro* studies and validated in additional cohorts. As in exposomics omics data integration can utilize pathway analysis leveraging the power of network and clustering methods to identify key biological disruptors.^(11,12)

Although there have been no previous EWAS/genome-wide association studies (GWAS), the power of combining environmental and genetic data has been exemplified in several studies. In one, a multistaged analysis was performed, beginning with a GWAS of over 8,000 individuals.⁽¹³⁾ Using linear regression adjusted for age and sex, single-nucleotide polymorphisms (SNPs) associated with increased betaine levels were identified. Following replication analysis in additional cohorts, a second stage was performed using unconditional logistic or multinomial regression, to determine which of these SNPs were also associated with increased coronary artery disease. SNPs from both the derivation and replication analyses were included following a meta-analysis performed separately for every SNP that passed criteria for quality control. A similar approach was applied to male and female subgroups in order to investigate sexually dimorphic effects. This approach demonstrated

that women with these SNPs had a decreased risk of coronary artery disease, suggesting sex-specific mechanisms of atherosclerosis. A similar staged approach could be used for integration of EWAS and GWAS data, either with classical statistical approaches and/or machine learning for variable selection. A paradigm for such an approach is depicted in Fig. 4.

Challenges and Opportunities

A detailed evaluation of the exposome is not without its challenges. The sheer volume of potential exposures is vast. To date, there are 146 million unique chemical substances that have been registered. Although many of these have low potential for human exposure, even 1% equates to almost 1.5 million compounds.

This also does not account for the innumerable infectious agents that also make up the exposome (i.e., the infectome). Exploration of the infectome requires specific measurement techniques as well as unique bioinformatic tools. In particular, there are emerging algorithms to identify viral integration sites and pathogens from next-generation sequencing, such as Virus Seq, sequence-based ultrarapid pathogen identification, and VirTect. Additional resources and bioinformatics tools for the analysis of the infectome have previously been well summarized.⁽¹⁴⁾ The exposome may also be confounded by numerous variables, including, but not limited to, sex, age, income/socioeconomic status, shared environment/household, and disease state or stage. Disease state is a particularly important consideration because case-control studies are limited by the fact that findings may be a consequence of, and not causative of, disease. Confounding can be dealt with at the level of study design as well as statistical analysis. In particular, multicentered studies (such as those within HELIX and EXPOsOMICS) will be required for derivation and validation across large samples. Longitudinal samples are also critical for determining inter- and intraindividual variability in exposomics, as well as which features are causative and which are secondary to disease processes (i.e., including subjects before and after disease development). Additionally, twin studies and evaluation of extreme phenotypes may also be instrumental. Finally, well-characterized cohorts are necessary to define appropriate phenomes to which all other omics are inevitably tied. Finally, the underlying assumption of the majority of exposome studies is that the exposure can be detected at the time of disease. Although this may not be true of every exposure, it may be true of exposures that are most critical to chronic disease development. In classic toxicology, high-dose exposure to a toxic chemical leads acutely to a particular phenotype, often with a stereotypical dose-response relationship (i.e., Tylenol or alcohol intoxication; Fig. 5A). In the setting of exposure *in utero* or during early development, either the exposure or associated metabolites/exposure-specific effects may be detectable in the affected individual (Fig. 5B). One of the best demonstrations of this concept is the transgenerational inheritance of stereotypical disease in rat offspring with maternal exposure to environmental toxicants.⁽¹⁵⁾ Offspring demonstrated patterns of epigenetic alterations specific to each exposure.⁽⁹⁾ Ideally, similar results could be observed in adults with chronic disease (Fig. 5C). Finally, Fig. 5D depicts a scenario in which bioaccumulation occurs (i.e., the accumulation of a chemical within an organism), leading to detection of the environmental toxicant long after the initial insult.

Past Studies on the Environmental Causes of Liver Disease

Both mechanistic and population studies have identified links between environmental exposures and liver disease. Although an exhaustive discussion of all the known environmental associations with liver disease is outside the scope of this review, these have been summarized in Table 3, some of which will be highlighted in the following sections.

THE HISTORY OF AFLATOXIN AND HEPATOCELLULAR CARCINOMA

The link between aflatoxin and hepatocellular carcinoma (HCC) represents a remarkable example of the benefits of exposure research. In the early 1970s, initial ecological studies found that aflatoxin contamination of food sources in Thailand and Africa was associated with high rates of HCC.⁽¹⁶⁾ Aflatoxin-derived DNA adducts (cancer-causing metabolites covalently bound to DNA) were identified in blood and urine in 1977 and 1981, respectively,⁽¹⁷⁾ providing biomarkers for the detection of aflatoxin exposure in individuals. It is believed that these DNA adducts interact with guanine residues in hepatocyte DNA, mutating the P53 tumor suppressor gene. Case-control and cohort studies demonstrated that patients with hepatitis B virus (HBV) infection and detectable urinary aflatoxin had a significantly increased risk of HCC (relative risk [RR], 59.0), compared to those with either positive urinary aflatoxin (RR, 3.4) or hepatitis B surface antigen positivity alone (RR, 7.0).^(18,19) Additional studies revealed that individuals with mutations in enzymes involved in the metabolism of aflatoxin are more likely to have detectable levels of serum aflatoxin adducts and up to a 77-fold increased risk of HCC, an example of gene-environment interaction.⁽²⁰⁾ Aflatoxin has also been associated with hypomethylation and up-regulation of numerous genes, including thioredoxin reductase 1, which decreases expression of enzymes necessary for the detoxification of aflatoxin, thereby enabling an increase in aflatoxin adducts.⁽²¹⁾ Other hypomethylated genes include proliferating cell nuclear antigen and cyclin K, both of which are involved in DNA repair.

NONALCOHOLIC FATTY LIVER DISEASE

A number of environmental toxicants have been associated with steatohepatitis (SH), leading to the proposed terms “toxicant-associated fatty liver disease” (TAFLD) and “toxicant-associated steatohepatitis” (TASH).⁽²²⁾ Although a comprehensive discussion of each agent is outside the scope of this review, this has thoroughly been reviewed.⁽²²⁾

Of increasing interest is the role of endocrine-disrupting chemicals in the development of nonalcoholic fatty liver disease. Endocrine-disrupting chemicals include bisphenol A (BPA), a ubiquitous chemical that is detectable in more than 90% of the U.S. population despite a half-life of only 4-5 hours. Rats exposed to BPA in isolation develop progressive hepatic steatosis⁽²³⁾; concomitant exposure to a high-fat diet leads to development of a more severe nonalcoholic steatohepatitis (NASH) phenotype, with increased inflammation and fibrosis, than those exposed to a high-fat diet alone.⁽²⁴⁾ Furthermore, male offspring of mice exposed to a maternal diet containing BPA had a statistically significant, dose-dependent development of HCC or hepatic adenoma.⁽²⁵⁾

Perfluorinated alkyl substances have a half-life of between 2 and 8 years and are used in products such as nonstick cookware, food packaging, and flame retardants. The most common of these are perfluorooctanesulphonate (PFOS) acid and perfluorooctanoic acid (PFOA). Mice exposed to PFOA and fed a high-fat diet developed more pronounced hepatocyte hypertrophy, lipid droplet accumulation, and inflammation than mice exposed to PFOA alone.⁽²⁶⁾ This suggests that PFOA might be one of the drivers that mediates the transformation from simple steatosis to NASH. Interestingly, a similar study evaluating the impact of PFOS demonstrated that both a 6-week high-fat diet and PFOS alone led to steatosis, but PFOS exposure with a high-fed diet seemed to attenuate the effect of either exposure alone.⁽²⁷⁾

Although there is evidence that environmental toxicants may lead to TAFLD and TASH and even precipitate metabolic syndrome, obesity itself may potentiate the hepatotoxic effect of certain chemicals, particularly lipophilic organic compounds.⁽²²⁾ Lipophilic environmental toxicants accumulate in adipose tissue and are subsequently released slowly into the blood, creating a persistent source that may last years.⁽²⁸⁾ One example is dichlorodiphenyldichloroethylene, a metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT). Although DDT was discontinued in the early 1970s, its metabolites are still detectable in serum samples of 75%-80% of the general population in the United States. Interestingly, DDT has been implicated in epigenetic changes associated with the possible transgenerational inheritance of obesity, with more than half of F3 generation rats developing obesity despite no direct exposure to DDT.⁽²²⁾

PRIMARY BILIARY CHOLANGITIS

Evidence has long pointed to an environmental trigger for primary biliary cholangitis (PBC).⁽²⁹⁾ Not only is there a lack of concordance for PBC in monozygotic twins, more than 95% of patients have detectable antimitochondrial antibodies. These autoantibodies cross-react with numerous microbial and xenobiotic antigens, including *Escherichia coli*, *Novosphingobium aromaticivorans*, and human betaretrovirus (HBRV), implicating these agents in the development of PBC.⁽²⁹⁾ Epidemiological studies have linked PBC with numerous other environmental factors, including recurrent urinary tract infections, hormone replacement therapy, nail polish, smoking, and even environmental toxicants (i.e., aromatic and halogenated hydrocarbons).^(29,30)

PRIMARY SCLEROSING CHOLANGITIS

By contrast, there have been relatively few environmental associations linked to primary sclerosing cholangitis (PSC). Similar to PBC, individuals with PSC appear to have a significant history of recurrent urinary tract infections.⁽³¹⁾ Smoking is negatively associated with PSC in patients with concomitant inflammatory bowel disease.⁽³¹⁾ Meanwhile, evaluation of the microbiome has generally demonstrated overall inconsistent results except for evidence of reduced alpha diversity in the majority of studies.⁽³²⁾

Future Directions

Exposome research promises to push the boundaries of precision medicine, and may help delineate the molecular subtypes of disease. Exposome studies in complex liver disease may be transformative, leading to insights that may result in changes to public health policy, disease screening, and surveillance as well as therapies. The potential implications of exposome research are outlined below:

- Evidence for creation of public health policy.
- Discovery of better screening tests for disease.
- Development of novel biomarkers for disease progression.
- Establishment of patient-directed therapy.

In order to achieve these goals, there is a growing interest in using existing as well as novel technology to measure both the exposome as well as its outcomes. Air pollution sensors have been paired with vehicles to measure and identify sources of air pollution, whereas silicone wristbands and satellites paired with spectrophotometry have been used to measure chemical exposures across populations.⁽³³⁾ On a more expansive scale, the Pediatric Research using Integrated Sensor Monitoring Systems (PRISMS) is developing both wearable and nonwearable sensors to monitor the internal and external exposome in children in their natural environment. Other groups are developing ingestible biosensors (a device that can measure chemical substances).⁽³⁴⁾ A thorough review of sensors has been reported elsewhere.⁽³⁵⁾

Conclusions

Complex disease develops as a result of biological responses in genetically susceptible individuals under a diversity of environmental pressures. Application of exposome approaches to study complex liver disease has the potential to improve understanding of disease risk and etiology. Moreover, such an approach can provide important insights regarding variability in symptoms and outcomes of liver disease among individuals.

Given the sheer magnitude of extant chemical entities in contemporary life, it is likely that numerous environmental toxicants with low effect sizes are involved in the evolution of liver disease. In order to understand the effects of gene $\times$ environment interactions, it is critical to incorporate multiomics analysis to better define the biological networks relevant in liver disease. These advances will enable discoveries of molecular pathways of liver disease, providing the framework for development of new biomarkers for screening and risk stratification as well as novel therapies.

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Abbreviations:

BPA	bisphenol A
DDT	dichlorodiphenyltrichloroethane
EWAS	exposome-wide associated studies
GWAS	genome-wide association studies
HCC	hepatocellular carcinoma
HRMS	high-resolution mass spectrometry
PBC	primary biliary cholangitis
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulphonate
PSC	primary sclerosing cholangitis
RR	relative risk
SNPs	single-nucleotide polymorphisms.

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FIG. 1.

External and internal components of the exposome. A diagram depicting examples of external and internal exposures. Notably, several exposures can be considered both internal as well as external and occur across multiple settings (i.e., air, water). The categories only serve to illustrate common examples.

FIG. 2.

Approaches to case-control exposome research design. (A) Bottom-up: Measurement of all external exposures allows for the identification of most significant disease-associated exposures. The internal dose of these exposures is measured in blood and biological specimens. (B) Top-down: Measurement of all internal exposures (including biological responses to exposures) is used to identify disease-associated exposures. External sources of these exposures are then identified.

FIG. 3.

The toxicological paradigm. Exposome research aims to characterize various phases of the toxicological paradigm (thin solid line) that may be altered by an individual's ability to metabolize and eliminate environmental exposures (dotted line), which leads to a variable internal dose as well as biologically effective dose (i.e., DNA and protein adducts). The biologically effective dose of an exposure then results in directly to modification of biological processes (i.e., DNA methylation, gene expression, and microbiome), which causes a preclinical effect. Over time, these preclinical changes accumulate and lead to eventual liver disease. Factors such as genetics, age, sex, nutrition, and concomitant disease can alter susceptibility at each step in the toxicological paradigm, thereby altering the risk of liver disease.

FIG. 4.

An untargeted approach to investigations of gene $\times$ environment interaction. Understanding gene $\times$ environment interactions will arguably require simultaneous studies of the genome, exposome, and other omics. Once candidates are identified, statistical and functional associations can be determined using classical statistics and/or machine learning techniques. These results would undergo validation and replication in human samples or could be tested in *in vivo* or *ex vivo* experiments using animal models, cell cultures, or organoids. These “big data” sets could be used to generate hypotheses for understanding disease pathogenesis and discovery of new disease biomarkers or therapies.

FIG. 5.

Examples of temporal relationships between exposures and disease phenotypes. (A) Classic toxicology: a single, high-dose exposure leading to disease. The exposure can be measured at the time of disease development (i.e., arsenic poisoning). (B) “Early-life” exposure: Multiple exposures in early life (i.e., preconception, *in utero*, or childhood) result in childhood disease. Exposures may be in relatively high doses or may simply lead to greater toxicity in a vulnerable state. (C) Adult exposure: Multiple exposures during childhood, adolescence, and/or adulthood result in adult disease. (D) Cumulative adult exposures: Exposure doses accumulate throughout adulthood, leading to adult disease and differences in rates of disease progression, symptoms, and response to treatment. Blue lines denote exposures, and red lines denote phenotype. The green area denotes multiple exposures rather than a single exposure. Abbreviations: E, exposome; P, disease phenotype.

TABLE 1.

Types of Omics Data

Epigenomics	Characterization of reversible modifications (i.e., DNA methylation) or DNA-associated proteins (i.e., histone acetylation)
Transcriptomics	Quantification of specific RNA transcript levels as well as identification of splice and editing sites
Metabolomics	Quantification of small molecules (<2,000 Da), such as amino acids, fatty acids, and carbohydrates
Proteomics	Quantification of peptides as well as analysis of peptide modifications and interactions
Immunomics	Characterization of immune system regulation and response to pathogens by combining proteomics with serological evaluation
Lipidomics	Quantification and quantitative analysis of lipids; can encompass both intra- and extracellular lipids; a subset of metabolomics
Adductomics	Quantification and identification of adducts, compounds that can bind to DNA, RNA, and protein, causing mutations and other downstream disruptions
Microbiomics	Quantification and characterization of all microorganisms in a community; often focused on bacteria, but may also include viruses and fungi

TABLE 2.**Bioinformatics Approaches to EWAS and Multiomics Integration**

Phase of Research	Categories	Tools
Variable selection	Statistical approaches	Generalized linear model Partial least squares regression (PLS) Canonical correlation analysis Least angle regression Gaussian graphical models Ridge regression ElasticNet regression Bayesian statistics Convolutional neural networks
Biological associations	Libraries	Gene Ontology Kyoto Encyclopedia of Genes and Genomes Reactome Comparative Toxicogenomics Database DAVID
	Identification/representation	Ingenuity Pathway Analysis Cytoscape Impala Mummichog
Risk estimates	Statistical approaches	Linear regression Logistic regression PLS Ridge regression Bayesian mixture models

Abbreviation: DAVID, database for annotation, visualization and integrated discovery.

TABLE 3.

Known Associations Between Environmental Exposures and Liver Disease

Liver Disease	Exposure Class	Exposure
Steatosis (macrovesicular)	Pharmaceutical agents	Didanosine, stavudine, zidovudine, 5-FU
	Environmental toxicants	Trichloroethylene, N,N-dimethylformamide, tetrachloroethylene, chloroform, vinyl chloride, volatile organic compounds (i.e., benzene, toluene, xylene, styrene)
Steatosis, SH, fibrosis	Pharmaceutical agents	Tamoxifen, methotrexate, amiodarone
	Environmental toxicants	Vinyl chloride, volatile organic compounds, tetrachloroethylene, chloroform, N,N-dimethylformamide
Fulminant hepatic failure	Infectious agents	Hepatitis B, hepatitis C, hepatitis D, hepatitis E, Epstein-Barr virus, herpes simplex virus-1 and -2, yellow fever, dengue, Q fever, <i>Plasmodium falciparum</i> , varicella-zoster virus, parvovirus B19, human herpesvirus 6
	Pharmaceutical agents	Chloroform, Tylenol, antituberculous (isoniazid ± rifampicin) Antimicrobials (TMP-SMX, nitrofurantoin, amoxicillin, azithromycin) Antifungals (terbinafine, ketoconazole, itraconazole) Anticonvulsants (phenytoin, valproic acid, carbamazepine) Herbal supplements (ma-huang, usnic acid) Nonsteroidal anti-inflammatory agents (diclofenac, etodolac) Others (disulfiram, prophyllthiouracil, methylidopa, gentuzumab)
PBC	Environmental toxicants	2-nitropropane, mercury, tetrachloroethylene, carbon tetrachloride
	Infectious agents	Recurrent urinary tract infections, <i>E. coli</i> , <i>N. aromaticivorans</i> , HBRV
	Environmental toxicants	Superfund sites, smoking, 2-nonyl alcohol, nail polish
PSC	Recurrent urinary tract infections	
	Cryptosporidium, intra-arterial chemotherapy <i>Ascaris lumbricoides</i> , <i>Clonorchis sinensis</i> , <i>Opisthorchis viverrini</i> , <i>Fasciola hepatica</i>	
Secondary sclerosing cholangitis	Minocycline, nitrofurantoin, hydralazine, methylidopa, statins, fenofibrate, interferon (alpha and beta), infliximab, adalimumab, etanercept	
Autoimmune hepatitis	HBV	
HCC	Infectious agents	Aflatoxin, vinyl chloride, carbon tetrachloride, polychlorinated biphenyls, dioxins and dioxin-like compounds, arsenic, tetrachloroethylene
	Environmental toxicants	

Abbreviations: 5-FU, 5-fluorouracil; SH, Steatohepatitis.