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Genetic basis of susceptibility to drug-induced liver injury: what have we learned and where do we go from here?

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Summary

Drug-induced liver injury (DILI) is a rare but severe adverse drug reaction that represents a major problem in pharmacotherapy, both clinically and economically. A considerable effort has been made over the past several decades to identify genetic factors that predispose to risk for DILI, and the pace of discovery has vastly increased over just the past several years. Here, we describe the major findings in DILI pharmacogenetics with an emphasis on the recent results of unbiased genome-wide association studies, and suggest some directions for future research in this area.

Keywords

DILI; hepatotoxicity; pharmacogenetics; pharmacogenomics; genome-wide association studies; GWAS; whole-genome sequencing

Drug-induced liver injury: Significance and early Genetic Investigation

Among the many unsolved problems in pharmacotherapy, arguably the most important relate to rare, or idiosyncratic, adverse drug events. Drug-induced liver injury (DILI) represents a particularly significant problem, both in terms of its clinical impact and its economic effects. DILI is currently the leading cause of acute liver failure in the US [1], and estimates of global incidence of DILI are as high as 13.9 per 100,000 patients per year [2]. Beyond its cost in terms of patient morbidity and mortality, DILI is among the most frequent reasons for late termination of drug development programs and regulatory actions including withdrawal of drugs from the market, resulting in large financial losses to the drug industry that are inevitably passed on to consumers [3].

For these reasons, much effort has been taken to identify causes of DILI and methods to predict its occurrence. Due to the idiosyncratic nature of DILI, it has long been suspected that risk for this adverse event is at least partly due to genetic factors. The earliest evidence in favor of this view came from targeted, hypothesis-driven genetic association studies conducted in the so-called “candidate gene era”, from which there emerged a view that DILI predisposition may be strongly influenced by genetic variation in “pharmacokinetic genes”, i.e. those involved in drug metabolism and transport. For example, isoniazid, which exhibits a large amount of interindividual variation in its metabolism due to functional

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polymorphism in the enzyme N-acetyltransferase 2 (NAT2), is among the most well studied drugs with respect to its potential to cause DILI. Slow acetylation of isoniazid has been fairly consistently associated with increased risk for DILI based on studies beginning decades ago, before the actual DNA sequence variant responsible for the slow acetylator phenotype was discovered, and this has been generally supported by more recent genetic association studies. Additionally, a number of other phase II as well as phase I drug metabolizing enzymes (CYP450s) and drug transporters (particularly the bile salt export pump, BSEP and the multidrug resistance associated protein 2, MRP2), have been nominally associated with DILI due to particular drugs or to DILI in general, regardless of drug (reviewed in [4, 5]).

In addition to genes involved in pharmacokinetics, immune-related genes such as those encoding the human leukocyte antigen (HLA) proteins and several cytokines have been nominally associated with risk for DILI [4]. That these genes were considered candidates for involvement in DILI reflects the recognition that some types of DILI progress after drug therapy is discontinued or occur in the presence of clinical features suggesting immune activation, such as fever and skin rash. The above studies, though potentially informative for the biology of DILI, suffer from a number of deficiencies that call into question their importance, and perhaps even their veracity [6]. These are no different from the shortcomings of the majority of human genetic studies of their time: 1) a focus on particular genes or gene variants that have a biologically plausible relationship to the trait, to the exclusion of all other genes; 2) a tendency toward small sample sizes; 3) a failure to account for population structure (i.e., genetic ancestry) and the possibility of confounding due to ancestry; and 4) a general lack of statistical rigor, and in particular a failure to account for multiple hypothesis testing even on the scale of the handful of variants tested in the typical candidate gene study. Because of these flaws, we should expect that, just like most of the contemporaneous genetic association studies of human disease, the published reports of DILI susceptibility genes from candidate gene studies will ultimately be found to contain a great many false positives.

DILI Genetics in the Genomic Era

In just the past 5 years, the technology and methodology of human genetic studies has matured to the point that comprehensive genome-wide investigation of the role of common single-nucleotide polymorphisms (SNPs) in essentially any measurable human trait can be performed at reasonable cost, and such genome-wide association studies (GWAS) address directly the criticisms above. The result has been a tremendous explosion in the number of secure associations between gene variants and a variety of complex human traits, including adverse drug responses such as DILI. Concurrently, due to the growing recognition that serious investigation of rare adverse drug reactions would require a significant investment in identifying and enrolling patients for genetic studies of DILI, a number of groups have been established with such efforts as a primary goal: these include the US-based, NIDDK-sponsored Drug-Induced Liver Injury Network (DILIN) [7], as well as international consortia in Europe and elsewhere (DILIGEN, iDILIC, EUDRAGENE, the Spanish DILI Cohort, and others) [8, 9].

With these resources having become available over the past few years, genome-wide association studies of DILI have yielded some additional insight into the biological underpinnings of this adverse event, beginning with the landmark finding in 2009 that liver injury due to the antibiotic flucloxacillin is strongly associated with the HLA-B*57:01 allele, with an odds ratio of approximately 80 for risk of flucloxacillin-DILI in HLA-B*57:01 carriers compared to non-carriers [10]. Subsequent GWAS that examined cases of DILI due to other causal drugs, including ximelegatran [11], lapatinib [12], lumiracoxib [13],

and most recently the antibiotic combination amoxicillin/clavulanate [14] have similarly shown strong associations between SNPs in the HLA region and risk for DILI. Although the particular variants associated with DILI risk have varied depending on which causal drug was being studied, for at least two drug pairs (ximelegatran and lapatinib; lumiracoxib and amoxicillin/clavulanate) the associated allele was shared across drugs, which suggested the possibility that there may be some variants or sets of variants that are risk factors for DILI generally, irrespective of the causal drug.

Recently, we and others performed a GWAS that included nearly 800 patients who had experienced DILI attributed to more than 200 different drugs, and over 3,000 control subjects, which represents the largest GWAS of DILI to date [15]. Although there was suggestive evidence for an association between a SNP in the Signal Transduction and Activator of Transcription 4 (STAT4) gene and hepatocellular injury no SNP showed clear association with DILI or any subtype of DILI at the genome-wide significance threshold. This study was well powered to detect moderate effects of common variants on risk for DILI in general (Odds Ratio > 2.0)

Conclusion

In evaluating the progress that has been made to date on understanding the genetics of predisposition to DILI, several points should be recognized. First, the more contemporary genome-wide approaches, which are unbiased toward any particular genes or pathways and have faithfully accounted for population structure and multiple hypothesis testing, appear to show a more significant role for common variants in genes related to immune response as opposed to genes involved in drug metabolism or transport. While it seems likely that variants in genes related to pharmacokinetics contribute to DILI risk, it appears that their effects may be too modest or the risk allele frequencies too rare to be detected in the typical GWAS. Second, although there may be some variants that predispose to DILI generally (i.e. without regard to the causal drug), their effects also appear to be too modest or the risk allele frequencies too rare to be detected by GWAS of all cause DILI cohorts. Finally, although several common variants have been strongly and unequivocally associated with risk for DILI through GWAS, it should be noted that their value as predictive markers, in particular their positive predictive value (PPV), is fairly low; given that DILI is a very rare occurrence and most studies to date have focused primarily or entirely on common variants, this is necessarily the case. Thus, we might imagine that certain common alleles may be required for DILI to occur in some instances, but their effects may be revealed only in the setting of some additional environmental or genetic modifier, such as a rare genetic variant that is usually found only in DILI cases and may have a much stronger PPV.

Future Perspective

Genetic studies of DILI registries have been successful in identifying unsuspected variants as risk factors and many additional insights can be expected as these registries grow and the analytical technology continues to advance. The experiences to date suggest several avenues of research that are likely to bear additional fruit. The fact that GWAS has been used successfully in identifying true genetic risk factors for DILI due to individual drugs, when reasonably sized samples of such DILI cases are available for study, would argue in favor of expanding efforts to identify new DILI cases due at least to the most represented drugs in the current DILI registries, such that drug-specific or class-specific studies may be performed with reasonable expectation of success. Associations identified can then be specifically tested in cases due to other drugs to search for common risk factors.

In addition to GWAS, more comprehensive and increasingly cost-effective methods of genome-wide interrogation are now maturing, with an eye toward identifying rare, functionally important gene variants that are expected to have a stronger influence on risk for disease, or similarly, for rare adverse drug events. Massively parallel (“next-generation”) sequencing studies of entire patient genomes, or of the whole exome (i.e. the protein coding region of the genome) have been used very successfully to identify rare functional variants underlying rare genetic diseases, and the same techniques are currently being used in the study of complex traits as well, on an increasingly large scale. Currently, there is much interest in taking advantage of these sequencing efforts to develop expanded genome-wide genotyping platforms to extend the GWAS principle to rarer and rarer variants, with an emphasis on putatively functional coding variants. Such “exome chip” experiments may in the short term reveal important genetic risk factors for complex traits such as DILI at relatively little cost; however, it should be recognized that such studies are limited by the fact that they can only interrogate variants that have already been discovered by previous sequencing studies and are therefore “blind” to variants that may be unique to a particular patient population such as DILI patients. Thus there will continue to be a serious advantage to comprehensive sequencing of DILI patients themselves, either whole-exome or whole-genome. Such studies will allow for identification of causal variants unique to those patients, and will also allow for genebased tests of multiple variants, which, if studies of Mendelian diseases are any guide, are likely to be a more powerful method of discovery than single-variant tests. While whole-exome sequencing currently provides a considerable cost advantage per sample sequenced compared to whole-genome sequencing, this is becoming less true as costs of whole-genome sequencing continue to decline. Whole-genome sequencing will ultimately be the method of choice for genetic discovery, as it allows for the detection of fine-scale structural variation as well as noncoding genetic variation, both of which are currently refractory to whole-exome sequencing. Apart from DNA sequence variation, little attention has been paid to date on the possible role of epigenetic factors, which may by themselves contribute to risk for DILI, or may modify effects of DNA sequence variants. It should be noted that associations with sequence variants may suggest nongenetic risk factors by identifying genes or pathways known to be under environmental influences, or that can be examined for epigenetic factors when liver biopsy tissue is available.

Finally, as the field moves more toward comprehensive whole-genome sequencing approaches and the ability to identify rare genetic variants, even variants that are “private” or unique to individual DILI cases, our approach toward evaluation of any putative risk variants must keep pace with our ability to discover them. In particular, the precise functional consequences of putative risk alleles will often need to be established empirically in order to help determine whether such variants are likely to be pathogenic. Additional emerging technologies will almost certainly be of immense help in this regard, including wholetranscriptome profiling by RNA-seq, proteomic and metabolomic approaches, and the currently very promising work that is being done with induced pluripotent stem cells (iPSC). There has already been considerable progress with culturing hepatocyte-like cells derived from iPSC [16], and it is conceivable that in the near future we may have the capacity to generate organotypic liver culture models, including hepatocytes and immune cells, from iPSC derived from patients who have experienced DILI. Such techniques will provide a previously unimaginable resource for studying the pathophysiology of DILI, and with the ability to genetically manipulate such cultured cells, will greatly aid in our understanding of the role of individual gene variants in DILI pathogenesis.

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Executive Summary

- Studies of DILI genetics in the “candidate gene era” suggested that DILI may be explained by variability in biotransformation and transmembrane transport of drugs, as well as innate immunity
- Genome-wide association studies suggest a strong role for common variants in immune-related genes in risk for DILI, particularly in the HLA region
- Current and future approaches including whole-genome sequencing may allow for the identification of rare, high-impact DILI risk variants
- Our ability to recognize such variants will likely depend on emerging technologies that will provide critical information regarding their functional consequences