A Supertype-based Approach to Improve Sensitivity of Co-amoxiclav HLA Associations

Summary

I propose to investigate the use of an HLA supertype-based approach to accurately identify patients susceptible to drug-induced liver injury (DILI) associated with the antibiotic co-amoxiclav. I hypothesize that by clustering HLA alleles into supertypes based on the similarity of their peptide binding cleft, we can increase the sensitivity of previously identified co-amoxiclav HLA associations, thereby improving the clinical usefulness of a genetic screen to identify patients at risk for DILI associated with this compound. I will test my hypothesis using HLA genotype data previously collected from patients who have experienced co-amoxiclav-induced liver injury and corresponding ancestry-matched population controls. A highly sensitive supertype association could be readily implemented as a clinical screen using currently available HLA genotyping technology. If successful, the application of this approach could be expanded beyond co-amoxiclav to other many other DILI-causing drugs.

Co-amoxiclav Induced Liver Injury

The combination of amoxicillin and clavulanate or co-amoxiclav (marketed under the trade name Augmentin) is an oral antibiotic widely used in the treatment of bacterial infections. According to the World Health Organization, it is an "essential medicine" – one of the most important medications needed in a basic health system [1]. However, co-amoxiclav is also currently by far the most common cause of non-acetaminophen DILI both in the United States and Europe [2]. Hepatic injury associated with co-amoxiclav is idiosyncratic in nature, occurring in ~1 in 2,500 prescriptions and typically presenting ~3 weeks after initiation of therapy (which is often after the course of antibiotic is completed). Recovery from the injury can be prolonged with significant morbidity, and in rare cases there can be a fatal outcome. Several genetic studies have indicated links with human leukocyte antigen (HLA) types, particularly the extended haplotype: DRB1*15:01-DRB5*01:01-DQB1*06:02 [3-6]. Genetic testing for these alleles provides a potential way to identify individuals that may be susceptible to co-amoxiclav-induced liver injury. However, these associations lack sensitivity (i.e. proportion of positives that are correctly identified), limiting their usefulness in a clinical screen for DILI susceptibility.

HLA Involvement in DILI Reactions

HLA alleles have emerged as significant risk factors for many idiosyncratic DILI causing drugs [7, 8]. The current thinking is that proteins encoded by these alleles present drug-associated neoantigens that simulate T-cell activation, thereby promoting an adaptive immune attack on the liver [9]. There are a variety of mechanisms proposed for the creation of neoantigens as a result of drug exposure (**Fig. 1**). The longest standing model is commonly referred to as the "hapten hypothesis" [10]. In this model, hepatocytes produce a reactive intermediate via metabolism of the parent drug. Covalent binding of this metabolite to liver proteins generates hapten-protein adducts that can be processed into a pool of chemically-modified peptides. These peptide neoantigens, when presented by HLA molecules, are recognized as "foreign" by T-cells and elicit an adaptive immune response. More recently, two hypotheses have emerged suggesting that T-cell mediated immune responses result from a drug (or its metabolites) interacting directly with immune receptors [11]. The "pharmacological interaction" or "p-i" model suggests that a drug binds noncovalently to either the T-cell receptor or HLA molecule and directly actives T-cells in a peptide-independent manner [12, 13]. The "altered peptide repertoire" model proposes that a drug binds to the peptide-binding pocket of the HLA molecule, thereby changing the chemistry of the binding cleft and altering the repertoire of self-peptides the HLA molecule presents [14-16]. This is plausible because only a small percentage of the peptides generated within cells bind to HLA molecules on the surface of cells and hence are recognized as self. When new peptides are presented, they can be seen as neoantigens by T-cells, even though they are not "new" to the cell. An "altered peptide repertoire" is the mechanism underlying abacavir hypersensitivity reactions, predominately impacting skin [16]. The association of specific HLA alleles with DILI risk therefore may be driven by the interaction of the offending drug with unique features of the HLA molecule, and in particular the peptide binding cleft.

HLA Supertypes

While HLA molecules are highly polymorphic, particularly in the region of peptide binding, they can be clustered into sets of molecules called "supertypes" that share homology in their peptide binding cleft [17, 18].

Therefore it is possible that associations of drug toxicities with specific HLA alleles may be conserved across other HLA molecules within the same supertype. Supertypes have been defined for both HLA class I and class II molecules based on either experimentally observed or computationally predicted peptide binding repertoires [19, 20]. HLA molecules that have a high degree of overlap in their peptide binding repertoires are considered to be the same supertype. A web-based tool called MHCcluster (http://www.cbs.dtu.dk/services/MHCcluster/) has been developed to functionally cluster MHC molecules based on their predicted binding specificity [21]. An example of clustering for representative HLA-B molecules is show in **Fig 2**.

HLA Supertype Approach Applied to Drug X

Recently, we investigated the possibility that taking an HLA supertype-based approach could improve the sensitivity of an HLA association identified for a proprietary drug, subsequently referred to as "drug X" (the pharmaceutical company sponsoring this work has agreed to publish these results but we have not yet received approval to share these data). We hypothesized that if the HLA association resulted from a direct interaction of the drug with the HLA binding pocket, as in the "altered peptide repertoire" model, HLA molecules within the same supertype could also interact with drug X and present neoantigen. Therefore, grouping HLA molecules into supertypes could improve the ability to correctly identify DILI susceptible patients. Genotyping of HLA molecules was performed using DNA from drug X-treated patients who did or did not experience DILI. The strongest association identified was with HLA-B*45:01, which was represented in 27% of cases compared to 0.01% of controls (p=0.006, odds ratio=26.7, sensitivity=0.23). We then classified all of the HLA alleles represented in our dataset into HLA supertypes, groups of HLA molecules with similar peptide binding preferences due to sequence conservation in the antigen-binding cleft, and reanalyzed the data using supertypes instead of individual alleles. Using this supertype approach, we determined that drug X-induced liver injury was found exclusively in individuals expressing HLA supertype B44 (p=0.0014, odds ratio=21.65, sensitivity=1). Using HLA supertype B44, we would be able to accurately predict 100% of low risk patients (negative predictive value), thus obviating the need for frequent monitoring in a large percentage of patients taking the drug. The sponsor is currently collecting data from a new clinical trial to confirm this finding for drug X.

Proposed Application of HLA Supertype Approach to Co-amoxiclav

I propose to apply a similar supertype-based approach to improve the sensitivity of HLA associations identified for co-amoxiclav. I have chosen co-amoxiclav as the initial target drug to investigate this approach for several reasons. First, it is a critical medicine and widely used. Second, it is the most common cause of non-acetaminophen DILI both in the United States and Europe. Finally, through collaboration with the US Drug Induced Liver Injury Network and the Severe Adverse Events Consortium established by my supervisor, Dr. Paul Watkins, I have access to HLA genotype data from over 200 patients who experienced co-amoxiclav-induced liver injury (by far the most of any drug represented in this database) and over 10,000 ancestry-matched population controls. As previously performed for drug X, I will assign supertypes to all subjects using HLA genotype data. I will then compare the results of association analysis performed using individual HLA alleles to analysis performed using HLA supertypes. A Fisher's exact test will be used to calculate association p-values and contingency tables will be generated to calculate the following performance characteristics: odds ratio, sensitivity, specificity, positive predictive value, and negative predictive value. I hypothesize that using HLA supertypes will result in improved sensitivity for identifying co-amoxiclav DILI cases when compared to using individual HLA alleles alone. Improving the ability to correctly identify DILI susceptible individuals (i.e. sensitivity) will yield a powerful diagnostic test to reduce adverse reactions in patients taking co-amoxiclav. HLA supertyping could be performed in a clinical setting using currently available genotyping technology by simply genotyping all alleles present in the supertype risk group. However to improve the utility of this approach, we would propose leveraging its success to drive the development of a new, more efficient "supertype assay" that takes advantage of the shared peptide binding properties of supertype alleles. Finally, the identification of a supertype association for co-amoxiclav would suggest that that prediction of risk for other HLA associated DILI reactions may be improved by analysis of HLA supertypes. I have full access to genotyping data from over 2,000 subjects with DILI due to a wide variety of medications. Therefore this same methodology could be applied to prevent adverse reactions to many other drugs.

Figures

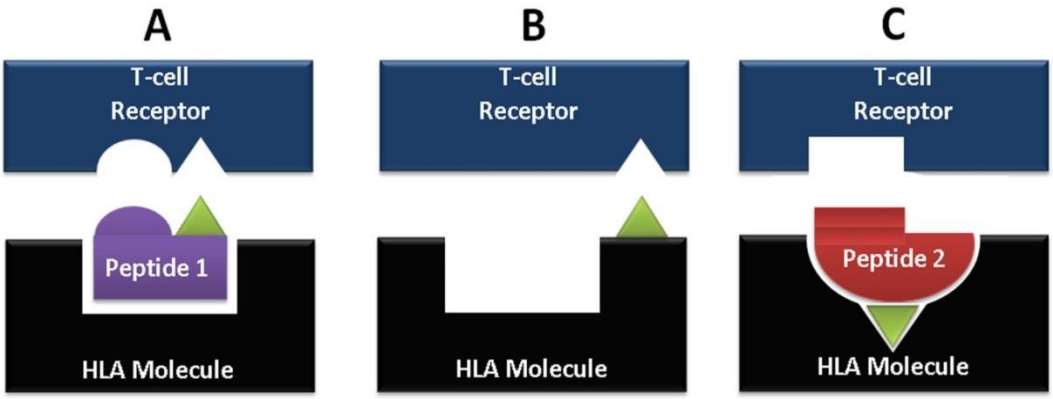

FIG. 1. Three models of neoantigen presentation by HLA molecules. (A) Hapten model: hepatocytes produce a reactive intermediate (represented in green) via metabolism of the parent drug. The covalent binding of this metabolite to liver proteins generates hapten-protein adducts that can be processed into a pool of chemically-modified peptides. When presented by a HLA molecules, these peptide neoantigens may be recognized as "foreign" and elicit an adaptive immune response. (B) Pharmacological interaction or p-i model: a drug (represented in green) binds noncovalently to either the T-cell receptor or HLA molecule and directly actives T-cells in a peptide-independent manner. (C) Altered peptide repertoire model: a drug (represented in green) binds to the peptide-binding pocket of the HLA molecule, thereby changing the chemistry of the binding cleft and altering the repertoire of the endogenous (unmodified) peptides it presents. When new peptides are presented, they may be seen as neoantigens by T-cells; they are not "new" to the cell but have never been presented by HLA molecules before.

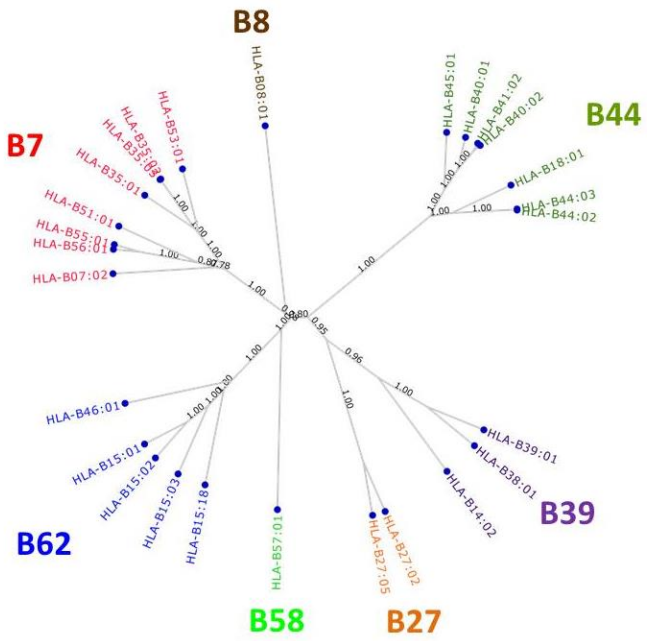

FIG. 2. Representative MHC specificity tree for HLA-B supertypes. HLA-B molecules are clustered into seven supertypes based similarities in their peptide binding repertoires. HLA-B supertypes are represented by different colors. Clustering was performed using a web-based tool called MHCcluster (http://www.cbs.dtu.dk/services/MHCcluster/).

References

1. WHO. *WHO Model List of Essential Medicines*. 2015; 19th:[Available from: http://www.who.int/medicines/publications/essentialmedicines/EML_2015_FINAL_amended_NOV2015.pdf?ua=1.
2. deLemos, A.S., et al., *Amoxicillin–Clavulanate-Induced Liver Injury.* Digestive diseases and sciences, 2016. **61**(8): p. 2406-2416.
3. Stephens, C., et al., *HLA Alleles Influence the Clinical Signature of Amoxicillin-Clavulanate Hepatotoxicity.* PLoS ONE, 2013. **8**(7): p. e68111.
4. Lucena, M.I., et al., *Susceptibility to Amoxicillin-Clavulanate-Induced Liver Injury is Influenced by Multiple HLA Class I and II Alleles.* Gastroenterology, 2011. **141**(1): p. 338-347.
5. Hautekeete, M.L., et al., *HLA association of amoxicillin-clavulanate–induced hepatitis.* Gastroenterology, 1999. **117**(5): p. 1181-1186.
6. Donaldson, P.T., et al., *Human leucocyte antigen class II genotype in susceptibility and resistance to co-amoxiclav-induced liver injury.* Journal of Hepatology, 2010. **53**(6): p. 1049-1053.
7. Urban, T.J., A.K. Daly, and G.P. Aithal, *Genetic Basis of Drug-Induced Liver Injury: Present and Future.* Semin Liver Dis, 2014. **34**(02): p. 123-133.
8. Grove, J.I. and G.P. Aithal, *Human leukocyte antigen genetic risk factors of drug-induced liver toxicology.* Expert Opinion on Drug Metabolism & Toxicology, 2015. **11**(3): p. 395-409.
9. Mosedale, M. and P.B. Watkins, *Drug-Induced Liver Injury: Advances in Mechanistic Understanding that will Inform Risk Management.* Clinical Pharmacology & Therapeutics, 2016.
10. Cho, T. and J. Uetrecht, *How Reactive Metabolites Induce an Immune Response That Sometimes Leads to an Idiosyncratic Drug Reaction.* Chemical Research in Toxicology, 2016.
11. White, K.D., et al., *Evolving models of the immunopathogenesis of T cell–mediated drug allergy: The role of host, pathogens, and drug response.* Journal of Allergy and Clinical Immunology, 2015. **136**(2): p. 219-234.
12. Pichler, W.J., et al., *Pharmacological Interaction of Drugs with Immune Receptors: The p-i Concept.* Allergology International, 2006. **55**(1): p. 17-25.
13. Pichler, W.J. and S. Watkins, *Interaction of Small Molecules with Specific Immune Receptors: The p-i Concept and its Consequences.* Current Immunology Reviews, 2014. **10**(1): p. 7-18.
14. Bharadwaj, M., et al., *Drug Hypersensitivity and Human Leukocyte Antigens of the Major Histocompatibility Complex.* Annual Review of Pharmacology and Toxicology, 2012. **52**(1): p. 401-431.
15. Pavlos, R., S. Mallal, and E. Phillips, *HLA and pharmacogenetics of drug hypersensitivity.* Pharmacogenomics, 2012. **13**(11): p. 1285-1306.
16. Norcross, M.A., et al., *Abacavir induces loading of novel self-peptides into HLA-B*57:01: an autoimmune model for HLA-associated drug hypersensitivity.* AIDS (London, England), 2012. **26**(11): p. F21-F29.
17. Sidney, J., et al., *HLA class I supertypes: a revised and updated classification.* BMC Immunology, 2008. **9**: p. 1-1.
18. Greenbaum, J., et al., *Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes.* Immunogenetics, 2011. **63**(6): p. 325-335.
19. Hoof, I., et al., *NetMHCpan, a method for MHC class I binding prediction beyond humans.* Immunogenetics, 2009. **61**(1): p. 1-13.
20. Nielsen, M., et al., *NetMHCIIpan-2.0 - Improved pan-specific HLA-DR predictions using a novel concurrent alignment and weight optimization training procedure.* Immunome Research, 2010. **6**: p. 9-9.
21. Thomsen, M., et al., *MHCcluster, a method for functional clustering of MHC molecules.* Immunogenetics, 2013. **65**(9): p. 655-665.