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Title: HLA-typing of patients with severe Adverse Drug Reactions

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Introduction:

Adverse Drug Reactions (ADRs) place a considerable socio-economic burden on the healthcare system as well as cause significant morbidity and mortality to patients. One of the primary aims in pharmacogenetics is to understand the genetic basis for such ADRs. The HLA region was the focus of this study as it is one of the most complex and gene dense regions in the human genome. Genes within this region play a critical role in the immune response as well as hypersensitivity reactions. Several ADRs have been characterized to be immunological in nature, and links between specific alleles and drugs such as Flucoxacillin (*HLA-B*57:01*), have already been established. The connection between HLA variants and ADRs are significant enough that mandatory testing has already been implemented for the drug Abacavir.

Aim: This project aims to investigate patterns in HLA variation that may confer risk of an ADR from taking two commonly prescribed medications; Angiotensin converting enzyme inhibitors (ACEi) (Angioedema) and Statins (Myalgia – muscle pain)

Impact: Though this project's focus is on ACEi and Statins specifically, exploring the associations between HLA variants and ADRs will help contribute to the overall understanding of the genetics of adverse reactions to medications. This knowledge might ultimately be used to help predict individual susceptibility and reduce morbidity and mortality of many ADRs.

Method:

Consented DNA samples from patients from the ADR cohorts were obtained from the Understanding Drug Reactions Using Genetic Sequencing (UDRUGS) study. Two different cohorts were included in this study, a cohort of seven patients with ACEi induced angioedema, and a cohort of eight patients with persistent statin myalgia.

The samples were analyzed for HLA variants by quantitative PCR, using the Roche Lightcycler 480. A haplotyping kit specific for the HLA region was obtained from TBG Biotechnology Group, and was optimized for use over the summer. Patient DNA samples from both cohorts had also previously undergone whole exome sequencing. In addition, HLA-typing software (Omixon®) was used to extract the HLA region from whole exome data enabling us to validate the results from the laboratory assay.

Due to the small sample size of the experimental cohort, the data was bootstrapped to get a statistical modelled answer comparable to a larger sample size. The mean allele frequency from the experimental cohort was then compared to allele frequency data from the Worldwide Allele Frequency database. Data was collected from individuals with European ethnicity as per the experimental cohort. Finally, the allele frequency means from the worldwide allele frequency database were compared to the study cohort and hypothesis testing was performed.

Results: Cohort data from both HLA-typing kits, and the software-analyzed whole exome data were found to be concordant with each other.

Several alleles were found to be enriched in the study cohorts in comparison to the database population (Fig. 1), and this difference was found to be statistically significant.

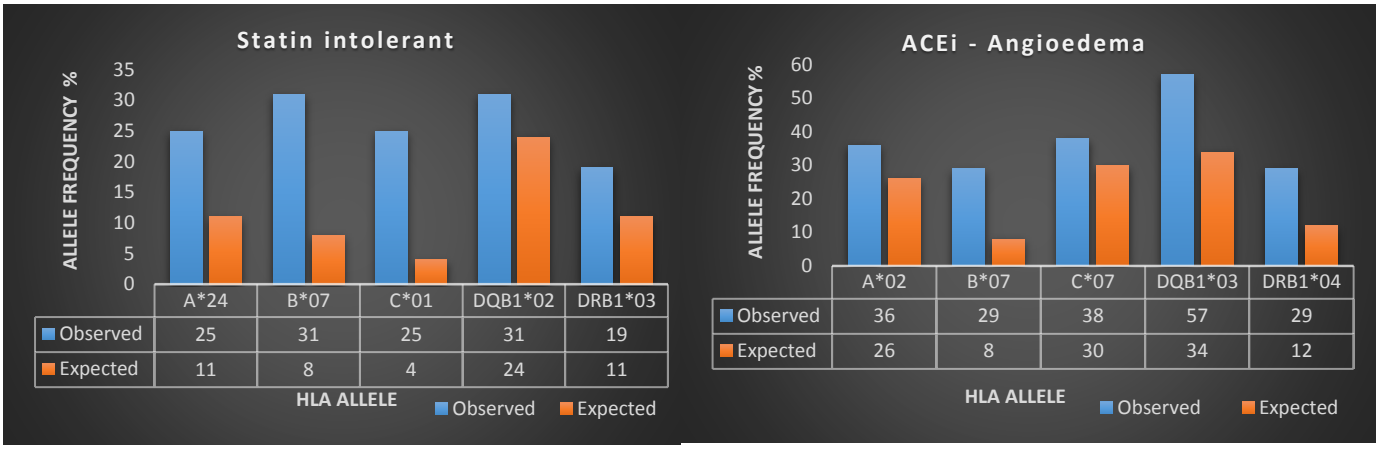

Figure 1: Comparison of study cohort allele frequencies and allele frequencies from the Allele Frequency database, for both Statin cohort and ACEi cohort.

Two alleles *HLA-B*07* and *HLA-C*01*, showed the greatest difference in allele frequencies between the study cohort and the database population, suggesting a possible association with these HLA variants and the presentation of statin intolerance and ACEi induced angioedema. Notably, allele *HLA-B*07* was found to be present in both the statin and ACEi cohort.

Conclusion:

This study has shown an association between specific HLA variants and the ADRs investigated. As this is a pilot study, this would need to be expanded and replicated with more patients in order to obtain clinically significant results.

*HLA-B*07* and *HLA-C*01* could be key alleles in the extension of this study. More focus would be placed on using techniques that can produce higher resolution genotype data, as this would help determine if there are particular variants of *B*07* and *C*01*, that are linked to the presentation of the investigated ADRs.

Interestingly, allele *B*07* is also present at a significant frequency in both the statin and ACEi cohorts. This presents another possible avenue of inquiry, to determine if this allele is common to other patients with different ADRs.