Analysis by PCR/Oligonucleotide Typing of HLA Class II Alleles in a Variety of Human Populations

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INTRODUCTION

The analysis of allele frequency distributions in various human populations can provide valuable anthropologic genetics information as well as useful data for forensics inferences about identity. One of the more controversial issues in the area of forensic inference, has been the question of population substructure and how this might effect the calculations of statistical weight (i.e., likelihood of a "random match") associated with an inclusionary result. The issue of substructure within the so-called census populations and the possibility that different subpopulations can have significantly different allele frequencies can be addressed most directly by determining the allele frequencies in many different population groups.

In the case of the HLA loci, given their critical role in mediating specific immune responses, population data can also help in estimating the likelihood of finding a matched donor for organ and bone marrow transplants as well as in understanding HLA-associated disease susceptibility. In addition, the pattern of allele distribution in different populations can provide valuable information for understanding the selective forces that have shaped the generation and maintenance of the HLA polymorphisms.

The HLA class I and class II loci are thought to be the most polymorphic genes (coding sequences) in mammalian biology. We have developed HLA PCR/oligonucleotide probe typing systems, in both the conventional dot blot (immobilized PCR product and labeled oligonucleotide probes) and the more convenient reverse dot blot (immobilized oligonucleotide probes and labeled PCR product) formats (1-5). These HLA class II typing systems analyze polymorphism at the second exon of the HLA-DRB1, -DQB1, -DQA1 (available commercially as the AmpliType® HLA-DQα test), and DPB1 loci. The HLA-DQα test has been used extensively in forensic casework beginning in 1986 (6). Recently we have also developed a reverse dot blot HLA-A typing system for analyzing polymorphism within the second and third exons (7). Our HLA class II typing systems have been used to determine the distribution of class II alleles and haplotypes in a variety of different populations. Here we present the results of our population studies and discuss the implications of the observed allele distributions for forensic analysis, particularly for DQA1.

RESULTS/DISCUSSION

Native Americans

Using PCR/oligonucleotide probe typing, we have analyzed the distribution of HLA-DRB1, -DQA1, -DQB1, and -DPB1 alleles as well as DRB1-DQB1 haplotypes in a variety of North Amerindian and South Amerindian groups. These studies revealed a striking lack of genetic diversity among all of these groups; a relatively small number of alleles and haplotypes have been identified, consistent with a population bottleneck during the colonization of the Americas by migration from Asia across the Beringia land bridge. For example, at the HLA-DQA1 (previously, the DQα) locus, with very few exceptions that are probably attributable to admixture, only the alleles *0301 (previously, α-3) and the *0401 and *0501 (grouped in the AmpliType HLA-DQα forensics test as α-4) are found in the groups studied. Similarly, for the other HLA class II loci, only a subset of the alleles found in other populations, were found in both North and South Amerindian groups. For this reason, genetic markers used for forensics inference, like the AmpliType HLA-DQα forensics test, are generally less informative (lower Power of Discrimination) in Amerindian populations than in other groups. Also, unlike the situation with other populations, distinguishing the subtypes of the DQα-4 type (4.1, 4.2, 4.3 or, more recently, *0501,*0401, *0601) can significantly increase the Power of Discrimination for this genetic marker in Amerindians. Future upgrades of the HLA-DQα reverse dot blot strip will contain additional probes for such subtyping.

As noted above, many DRB1 alleles are absent among Amerindians. A striking exception are the DR4 subtypes, where in Amerindians overall, several different DRB1 alleles were found. It is not clear whether many DR4 DRB1 alleles survived the putative population bottleneck that occurred during the migration from Asia or whether some of these DR4 subtypes were generated from the founding DR4 DRB1 alleles **after** colonization of the Americas. In the Cayapa Indians of Ecuador, a new DR8 allele, DRB1*08042, was identified (8). The finding of this new allele, presumably derived by point mutation from DRB1 *0802 within codon 86 (Gly-86 to Val-86) implicates the operation of selective forces favoring DRβ position 86 variants, because in Africa, DRB1*0802 gave rise to the same DRβ -86 variant (*08041) by another mechanism. Many DRB1 alleles that differ only at position 86 are found in many human populations and, recently, evidence indicating that the spectrum of peptides bound in the peptide-binding cleft of these molecules is different has been reported.

Although, like the other class II loci, only a few alleles at the DPB1 locus are found among most Amerindian groups, the allele frequency distributions can differ dramatically. For example, the frequency of DPB1*1401 is about 50% among the Cayapa Indians and is high among other Amerindian tribes in Ecuador and Colombia (8); this allele, however, is very rare (i.e., less than 3%) in most other Amerindian groups as well as all other human populations studied.

Some DRB1-DQB1 haplotypes appear to be unique to Native American populations; DRB1*1602, DQB1*0301 has been found in high frequency in all Amerindian groups studied and, to our knowledge, in no other groups. This marker, as well as others that are very frequent among Amerindians and absent or rare in other populations could serve as a population specific marker.

PACIFIC/ASIAN POPULATIONS

Recently, we have analyzed the class II polymorphism among two different Filipino populations as well as Indonesians, Malaysian, Papua New Guinea, and Hawaiian populations. One of the Filipino populations was sampled by using buccal swab samples collected at a high-school class reunion and a bowling alley (9). Several of these populations were characterized by a very high frequency of DR2 (and the haplotype associated DQA1*0102, or α1.2). However, the DR2 subtype at DRB1 was often different as was the DRB1-DQB1 haplotype in the different populations. Overall, these groups had a significantly different allele frequency distribution from previously reported Asian groups. In general, the differences between different Asian/Pacific groups was much greater than observed between different European Caucasian groups. This study also revealed several DRB1-DQB1 haplotypes that appeared to be unique to a given group. For example, DRB1*0405-DQB1*0503 was found only in Filipinos. DRB1*1502-DQB1*0502 was found at a very high frequency among Filipinos and was frequent among Indonesians but absent from the other populations studied.

SUMMARY/CONCLUSIONS

Forensics inference from genetic typing results is dependent on population data bases. Moreover, some of the issues associated with potential population substructure and assumptions of statistical independence (Hardy-Weinberg equilibrium and linkage equilibrium) can be addressed by collecting data from a variety of different populations. The extensive polymorphism at the HLA class II loci make these loci very informative markers for analyzing allele and haplotype frequency data. The data discussed here indicate that, for the HLA class II loci, Native Americans show significantly less genetic diversity than other populations studied. The study of Asian populations suggests that the allele frequency differences between these groups are much greater than between different groups of European Caucasians. The analysis of DRB1-DQB1 haplotypes as well as DPB1 alleles suggests that some of these (e.g., DRB1*1602, DQB1*0301 for Native Americans or DRB1*0405, DQB1*0503 for Filipinos) may serve as population specific markers.

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