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Analysis of HLA Class II Haplotypes in the Cayapa Indians of Ecuador: A Novel DRB1 Allele Reveals Evidence for Convergent Evolution and Balancing Selection at Position 86

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Summary

PCR amplification, oligonucleotide probe typing, and sequencing were used to analyze the HLA class II loci (DRB1, DQA1, DQB1, and DPB1) of an isolated South Amerindian tribe. Here we report HLA class II variation, including the identification of a new DRB1 allele, several novel DR/DQ haplotypes, and an unusual distribution of DPB1 alleles, among the Cayapa Indians (N=100) of Ecuador. A general reduction of HLA class II allelic variation in the Cayapa is consistent with a population bottleneck during the colonization of the Americas. The new Cayapa DRB1 allele, DRB1*08042, which arose by a G→T point mutation in the parental DRB1*0802, contains a novel Val codon (GTT) at position 86. The generation of DRB1*08042 (Val-86) from DRB1*0802 (Gly-86) in the Cayapa, by a different mechanism than the (GT→TG) change in the creation of DRB1*08041 (Val-86) from DRB1*0802 in Africa, implicates selection in the convergent evolution of position 86 DRβ variants. The DRB1*08042 allele has not been found in >1,800 Amerindian haplotypes and thus presumably arose after the Cayapa separated from other South American Amerindians. Selection pressure for increased haplotype diversity can be inferred in the generation and maintenance of three new DRB1*08042 haplotypes and several novel DR/DQ haplotypes in this population. The DPB1 allelic distribution in the Cayapa is also extraordinary, with two alleles, DPB1*1401, a very rare allele in North American Amerindian populations, and DPB1*0402, the most common Amerindian DPB1 allele, constituting 89% of the Cayapa DPB1. These data are consistent with the postulated rapid rate of evolution as noted for the class I HLA-B locus of other South American Indians.

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Introduction

The evolution of the extensive polymorphism in the class I and class II loci of the mammalian major histocompatibility complex (MHC; human leukocyte antigen [HLA] in humans) has been the subject of considerable controversy. The sequence-defined polymorphism in the HLA class II genes is located primarily in the second exon, which encodes the peptide binding groove of the cell surface heterodimer on antigen-presenting cells. Some authors argue that most of the MHC polymorphism is very old, transmitted from ancestral species, and that the rate of evolutionary change is not substantially different from that of other mammalian genes (Klein et al. 1991, 1993). Others suggest that, because of selection, the rate of diversification is relatively rapid (Howard 1992). The recent identification of new HLA-B variants in South American Indian tribes (Belich et al. 1992; Watkins et al. 1992) is consistent with this latter view. This inference is based on the assumption that genetic variants unique to Native American populations arose after the colonization of the Americas by Asian groups migrating across the Beringian land bridge some 10,000–40,000 years ago. Here, we report HLA class II variation, including identification of a new HLA-DRB1 allele, several unusual DR/DQ haplotypes, and a unique distribution of DPB1 alleles among the Cayapa Indians (N=100) of Ecuador.

The Cayapa Indians belong to the Chibchan-Paezan linguistic branch of the Amerind family, one of the three major subdivisions (Amerind, Nadene, and Aleut-Eskimo) of Native American languages, which may correspond to the postulated three waves of migration of Asiatic people to the Americas (Williams et al. 1985; Greenberg et al. 1986). Migrating via routes either from Panama or from the Caribbean Islands via Florida, populations were dispersed throughout South America by 12,000 B.C. (Salzano and Callegari-Jacques 1988). Although the origin of the Cayapa is highly debated, they are believed to be the first inhabitants of Ecuador (Barriga Lopez 1987). Further, evidence from traditional red-cell antigen markers indicates extant genetic isolation in the Cayapa (Rickards et al. 1994; Scacchi et al. 1994).

The nucleotide sequence of the new Cayapa DRB1 allele, *08042, which arose by a G→T point mutation, im-

plicates selection in the convergent evolution of position 86 DRB variants. In addition, DPB1*1401, a very rare allele in most other populations studied, is found in high frequency in the Cayapa, a finding also consistent with strong selection pressures. Our data also suggest that these novel allele and haplotypic patterns found in the Cayapa arose relatively recently, after the separation of North and South American Amerindians (~10,000 years ago); this finding is consistent with the relatively rapid rate of evolution proposed for the MHC class I, B locus diversity observed in other South Amerindians (Belich et al. 1992; Watkins et al. 1992).

Material and Methods

Samples

Blood samples were collected from 100 unrelated members of the Cayapa Indian tribe in Ecuador; none are presumed to be first-degree relatives. Genomic DNA was extracted from peripheral blood leukocytes of 100 individuals by using an inorganic salting-out procedure (Miller et al. 1988).

DR/DQ Haplotype Analysis

HLA class II haplotypes (DRB1-DQA1-DQB1) were inferred from established linkage disequilibrium patterns (Begovich et al. 1992) or were unequivocally determined by homozygosity. Haplotypes were corroborated by reestimating using the computer program of Baur and Danilov (1980), and two locus linkage disequilibrium values were measured for this population, to be reported in a forthcoming article (E. A. Titus-Trachtenberg, H. A. Erlich, O. Rickards, G. F. DeStefano, and W. Klitz, unpublished observations).

HLA Class II Typing

Genomic DNA was amplified using the PCR (Mullis and Faloona 1987; Saiki et al. 1988) and was typed using sequence- and allele-specific oligonucleotide probe methods for DRB1 (Scharf et al. 1991), DQA1 (Saiki et al. 1989), DQB1 (Bugawan et al. 1990), and DPB1 (Bugawan and Erlich 1991; Moonsamy et al. 1992, in press). Genomic DNA from the anomalous DRB1*08 samples were amplified with DRB1 YSTG primers RAP59 (Apple and Erlich 1992) and AB60 (5'CCGAATTCCGCTGCACTGTGAAGCTCTC3') by using the following profile: denaturation at 95°C for 1 min, annealing at 65°C for 1 min, extension at 70°C for 30 s; 35 cycles.

In our analysis of 200 HLA class II haplotypes analyzed at the DRB1 locus, nine samples were identified with a novel sequence-specific probe hybridization pattern on one chromosome; these samples failed to hybridize to the three existing position 86 probes CRX57 (V), CRX56 (G), and DRB181 (AV), suggesting a novel polymorphism and allele. With one homozygote exception, the homologues for the remaining eight anomalous samples carried DRB1*0411, *1402, *0404, *0901, or *0802 alleles.

Table 1

HLA Class II Allele Frequencies in the Cayapa (2*N* = 200)

Allele	No. (%)	Allele	No. (%)
DRB1:		DR9:	
DR2:		0901	40 (20.0)
1503	1 (.5)	DQA1:	
1602	18 (9.0)	0310	113 (56.5)
DR4:		0401	29 (14.5)
0403	1 (.5)	0501	58 (29.0)
0404	8 (4.0)	DQB1:	
0407	55 (27.5)	0301	56 (28.0)
0408	1 (.5)	0302	83 (41.5)
0410	1 (.5)	0303	39 (19.5)
0411	7 (3.5)	0402	22 (11.0)
DR5:		DPB1:	
1102	2 (1.0)	0201	7 (3.5)
DR6:		0301	2 (1.0)
1402	39 (19.5)	0401	2 (1.0)
DR8:		0402	79 (39.5)
0802	17 (8.5)	1301	11 (5.5)
08042	10 (5.0)	1401	99 (49.5)

*Sequencing the Novel DRB1 *08 Allele*

Samples with novel DRB1 YSTG probe hybridization patterns were amplified using primers CRX28 and AB60 (DRB1 gene) (Begovich et al. 1992) and primers RAP59 (Apple and Erlich 1992) and AB60 (5'CCGAATTCCGCTGCACTGTGAAGCTCTC3') by using the following profile: denaturation at 95°C 1 min, annealing at 65°C for 1 min, extension at 70°C for 30 s; 35 cycles. DRB1 sequences, amplified from 6 of the 10 samples with an unusual probe reactivity pattern, were cloned into M13mp19 (Scharf et al. 1986), and the nucleotide sequence was confirmed from 6 independent clones from each sample (Sanger et al. 1977).

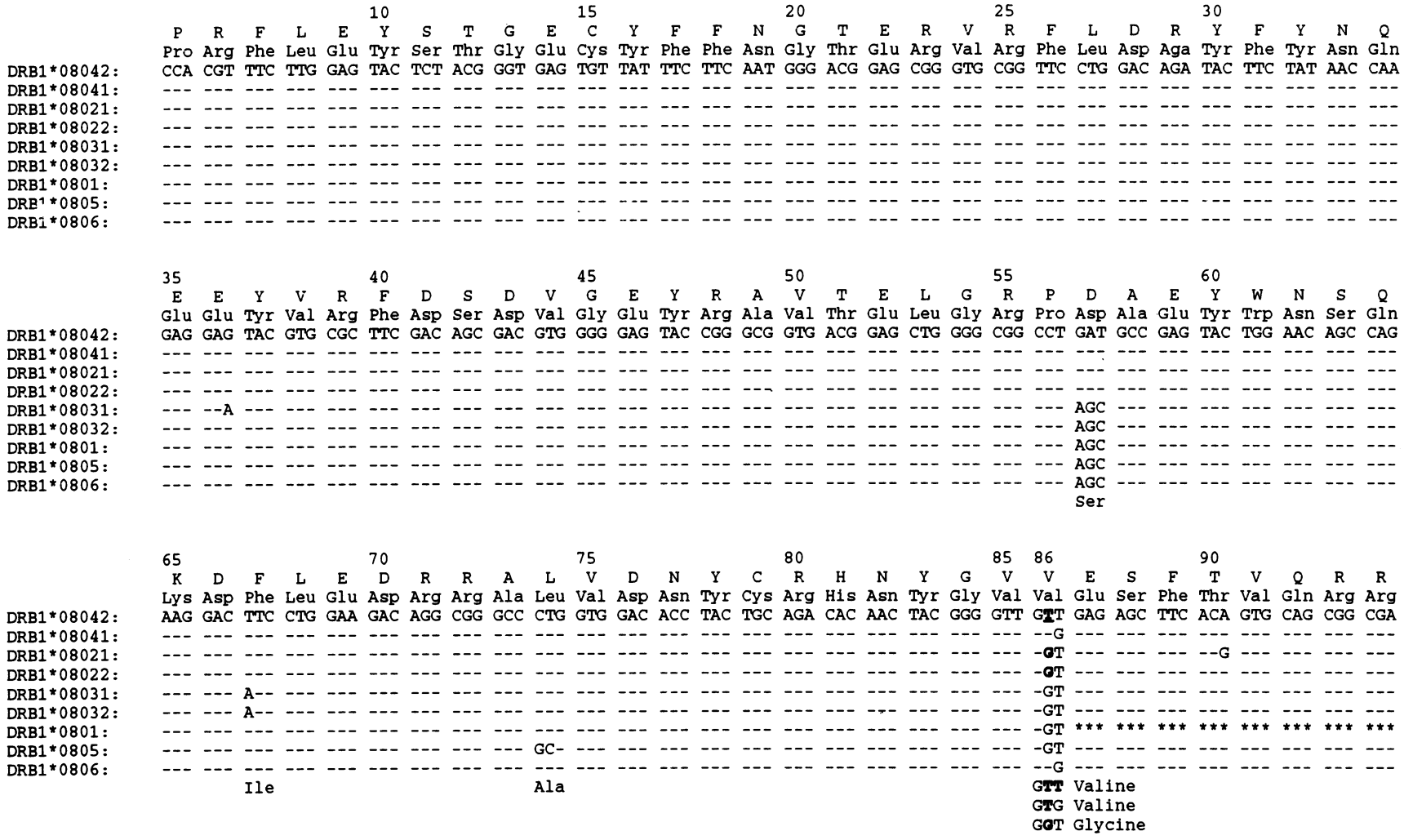
*DRB1 *08042 Sequence-specific Oligonucleotide (SSO)*

An SSO probe (ET1062;CGGGGTTGTTGAGAGCT) was hybridized (2 × SSPE/0.5% SDS for 30 min at 42°C, then washed in 0.2 × SSPE/0.1% SDS for 15 min at 42°C) to DNA amplified from genomic templates that had been immobilized on a Biodyne filter (Scharf et al. 1986). Hybridization of all of the nine original samples corroborated the presence and sequence of the new allele.

Results

HLA Class II Allele Frequencies

HLA class II allele frequencies for the Cayapa Indians are summarized in table 1. Of the 13 DRB1 alleles found in the Cayapa, 4 of them (*1602, *0802, *1402, and *0407) are frequent and are common to all Native American


10 15 20 25 30
P R F L E Y S T G E C Y F F N G T E R V R F L D R Y F Y N Q
Pro Arg Phe Leu Glu Tyr Ser Thr Gly Glu Cys Tyr Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Aga Tyr Phe Tyr Asn Gln
DRB1*08042: CCA CGT TTC TTG GAG TAC TCT ACG GGT GAG TGT TAT TTC TTC AAT GGG ACG GAG CGG GTG CGG TTC CTG GAC AGA TAC TTC TAT AAC CAA
DRB1*08041:
DRB1*08021:
DRB1*08022:
DRB1*08031:
DRB1*08032:
DRB1*0801:
DRB1*0805:
DRB1*0806:
35 40 45 50 55 60
E E Y V R F D S D V G E Y R A V T E L G R P D A E Y W N S Q
Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln
DRB1*08042: GAG GAG TAC GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG GCG GTG ACG GAG CTG GGG CGG CCT GAT GCC GAG TAC TGG AAC AGC CAG
DRB1*08041:
DRB1*08021:
DRB1*08022:
DRB1*08031: --A AGC
DRB1*08032: AGC
DRB1*0801: AGC
DRB1*0805: AGC
DRB1*0806: AGC
Ser
65 70 75 80 85 86 90
K D F L E D R R A L V D N Y C R H N Y G V V E S F T V Q R R
Lys Asp Phe Leu Glu Asp Arg Arg Ala Leu Val Asp Asn Tyr Cys Arg His Asn Tyr Gly Val Val Glu Ser Phe Thr Val Gln Arg Arg
DRB1*08042: AAG GAC TTC CTG GAA GAC AGG CGG GCC CTG GTG GAC ACC TAC TGC AGA CAC AAC TAC GGG GTT GTT GAG AGC TTC ACA GTG CAG CGG CGA
DRB1*08041: --G
DRB1*08021: -GT --G
DRB1*08022: -GT
DRB1*08031: A-- -GT
DRB1*08032: A-- -GT
DRB1*0801: -GT *** *** *** *** *** *** *** ***
DRB1*0805: GC- -GT
DRB1*0806: --G
Ile Ala GTT Valine
GTG Valine
GGT Glycine

groups sampled thus far (table 1; R. C. Castro and H. A. Erlich, unpublished data; E. A. Titus-Trachtenberg, G. Keyeux, J. Bernal, and H. A. Erlich, unpublished data) (Imanishi et al. 1992; Cerna et al. 1993). Together with DR9, an allele common in Asian groups (Imanishi et al. 1992) but rare in most Native American groups, these five DRB1 alleles constitute >90% of all DRB1 alleles among the Cayapa. Many DRB1 alleles (e.g., DR1, DR3, and DR7), common in other populations, are absent from this and other Native American groups analyzed to date, suggesting a population bottleneck and a consequent reduction in DRB1 allele diversity during the colonization of the Americas. Elimination of these alleles, by natural selection (e.g., susceptibility to infectious disease) following colonization of the Americas, remains a possibility but seems less likely, since these alleles are absent in virtually all North and South Amerindian groups. Additionally, in the Cayapa, no DQA1*0101-3, DQA1*0201, DQB1*0501-4, or DQB1*0601-5 alleles were found.

*Identification of the DRB1 *08042 Allele and Associated Haplotypes*

HLA class II analysis of the Cayapa Indians, using PCR and oligonucleotide probe typing, revealed a new DRB1*08 allele (fig. 1). Designated "DRB1*08042" (the name was officially assigned by the WHO Nomenclature Committee in February 1993; the nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number L10402), this allele was found at a relatively high frequency (f=.05) in this population (table 1). Probe hybridization patterns with DNA amplified from 10 samples indicated that the putative new allele was related to DRB1*0802 or DRB1*0804; however, the amplified DNA failed to hybridize to any of the SSO (Val and Gly) probes to the highly conserved, normally dimorphic position 86 codon, suggesting that the new alleles encoded either a novel amino acid residue or silent polymorphism at position 86. Sequence analysis of M13 clones containing the DRB1*0802 allele revealed a novel (GTT) Val codon (fig. 1).

Although the new Cayapa DRB1*08042 allele presumably arose relatively recently (i.e., after the migration of Native Americans into South America), this allele is found on three different DR/DQ haplotypes, with DRB1*08042-DQA1*0401 in association primarily with DQB1*0402 (8/10), but also with DQB1*0301 (1/10) and DQB1*0302 (1/10). The DRB1*08042 allele probably originated by point mutation from the presumptive parental DRB1*0802-DQA1*0401-DQB1*0402 haplotype, with subsequent recombination between DQA1 and DQB1 to generate the other uncommon DRB1*08042 haplotypes.

Unique Class II Haplotypes

Although the reduced number of common DRB1, DQA1, DQB1, and DPB1 alleles (see below) among the Cayapa is consistent with a population bottleneck, the diversity of DR/DQ haplotypes (n=24) (table 2) suggests that selection for new haplotypes generated by recombination between DQA1 and DQB1, or between DQA1 and DRB1, may be operating. Class II haplotypes (DRB1-DQA1-DQB1) for the Cayapa were inferred by homozygosity or from linkage disequilibrium patterns between these loci (Begovich et al. 1992). In general, the number of different DQA1, DQB1, and DRB1 alleles in the Cayapa is only about ⅓, whereas the number of different DR/DQ haplotypes is about ¾ of those found in a recent Caucasian population study (Begovich et al. 1992).

In addition to the novel haplotypes associated with the new allele, unusual DR/DQ combinations were also found in two DRB1*0802-DQA1*0401 haplotypes carrying either DQB1*0302 or DQB1*0303 alleles. Unique DR2 (DRB1*1503-DQA1*0301-DQB1*0302), DR9 (DRB1*0901-DQA1*0301-DQB1*0302), and DR14 (DRB1*1402-DQA1*0501-DQB1*0303) samples, as well as three DR4 (DRB1*0407, *0408 and *0410) samples, found in novel association with DQA1*0401-DQB1*0302, were also discovered.

HLA-DPB1 Allele Distribution

PCR/SSO probe analysis of the DPB1 locus in the Cayapa revealed an unusual distribution of alleles, with DPB1*0402 (f=.395) and DPB1*1401 (f=.5) the predominant alleles (table 1). DPB1*0402 is the most common allele in all other Native American populations studied to date (R. C. Castro and H. A. Erlich, unpublished data; S. Mack, personal communication) (Imanishi et al. 1992; Cerna et al. 1993). However, DPB1*1401 is found in low to very low frequencies in all populations studied, including Brazilian and Argentine tribes (f=.01 to f=.061) (Cerna et al. 1993), Southeast Asians (f=.049) (Imanishi et al. 1992), and North Amerindians (only one sample found) (R. C. Castro, unpublished observations), and is infrequent in all other populations analyzed (f~.01) (Imanishi et al. 1992). The very low frequency of DPB1*1401 in other populations, in combination with a very strong linkage disequilibrium observed between DPB1*1401 and the most frequent DRB1 allele *0407, as well as the new DRB1 allele

Figure 1 Nucleotide sequence of the second exon of DRB1*08042 allele in comparison with DRB1*08041 and DRB1*0801 through DRB1*0806 (Marsh and Bodmer 1991; Apple and Erlich 1992; Eberle and Baxter-Lowe 1992). A dash indicates identity with the DRB1*08042 sequence, and an asterisk indicates that the sequence has yet to be determined. Numbers above the nucleotide sequence refer to the corresponding amino acid in the protein sequence. Boldface letters and underlined letters indicate the nucleotide changes that are present.

Table 2

HLA DRB1-DQA1-DQB1 Haplotype Frequencies in the Cayapa Indians

Haplotype	No. (%)	Haplotype	No. (%)
DR2:		DR6:	
1503-0301-0302	1 (.5)	1402-0501-0301	36 (18.0)
1602-0501-0301	16 (8.0)	-0301-0302	1 (.5)
-0302	2 (1.0)	-0501-0301	1 (.5)
DR4:		-0501-0303	1 (.5)
0403-0301-0302	1 (.5)	DR8:	
0404-0301-0302	8 (4.0)	0802-0401-0302	2 (1.0)
0407-0301-0302	54 (27.0)	-0303	1 (.5)
-0401-0302	1 (.5)	-0402	14 (7.0)
0408-0401-0302	1 (.5)	08042-0401-0301	1 (.5)
0410-0301-0302	1 (.5)	-0302	1 (.5)
0411-0301-0301	1 (.5)	-0402	8 (4.0)
-0302	6 (3.0)	DR9:	
DR5:		0901-0301-0302	3 (1.5)
1102-0501-0301	2 (1.0)	-0303	37 (18.5)
		Total	200

*08042 (E. A. Titus-Trachtenberg, H. A. Erlich, O. Rickards, G. F. DeStefano and W. Klitz, unpublished data), are consistent with strong selection for a particular gene or set of genes on these haplotypes in the Cayapa.

Discussion

Analysis of the HLA class II alleles in the Cayapa Indians of Ecuador reveals a loss of diversity of class II alleles, similar to the restricted polymorphism noted for other Amerindian populations. In the Cayapa and other Amerindian populations examined to date, the DQA*01, DQA*02, DQB1*05, and DQB1*06 lineages are absent, as are the DRB1*01, *03, *07 and *10 lineages (Imanishi et al. 1992; R. C. Castro and H. A. Erlich, unpublished observations). This reduction in class II diversity is consistent with a population bottleneck of the putative ancestral Asian population, which migrated over the Bering land bridge to North America 10,000–40,000 years ago. In addition, the absence of European admixture in the Cayapa population is consistent with their history of genetic isolation, despite the possibility for admixture during the last ~500 years (Barriga Lopez 1987).

The appearance of a new allele, DRB1*08042, that presumably arose after the colonization of South America (~10,000–20,000 years ago), supports the notion of a relatively rapid rate of evolution in the class II region. The new allele, DRB1*08042, which encodes Val at position 86, appears to have been generated by a single transversion event ([Gly] GGT→GTT [Val]) within codon 86 of the putative parental DRB1*0802 allele. (In principle, DRB1*08042 could have arisen by point mutation from DRB1*08041; this alternative pathway seems much less likely, however, because DRB1*08041 has been found only in Africa and African Americans [E. Titus-Trachtenberg, unpublished data] and not among Amerindians. Moreover, a silent substitution converting DRB1*08041 to DRB1*08042 would not change the structural properties of the DRB molecule and hence would not be selected for.) The DRB1*08042 allele appears to be unique to the Cayapa. The DRB1*0802 allele, however, is a common and presumably the ancestral Amerindian DRB1*08 allele; it is the only DRB1*08 allele found in a survey of 490 North Amerindian haplotypes (table 2; Imanishi et al. 1992; R. C. Castro and H. A. Erlich, unpublished data). Moreover, DRB1*0802 was the only DRB1*08 allele found, with two exceptions (see below) in several other South Amerindian populations, including 680 Colombian (E. A. Titus-Trachtenberg, G. Keyeux, J. Bernal, and H. A. Erlich, unpublished data) and 646 Brazilian and Argentine Indian haplotypes (Imanishi et al. 1992; Cerna et al. 1993; S. Mack, personal communication). The presence of the new allele in 10 of 200 (f=.05) Cayapa DRB1 alleles suggests that this novel DRB1 allele has spread and has been maintained in this population by either selection or genetic drift.

As with many other DRB1 alleles that have arisen as position 86 variants (fig. 2) (Apple and Erlich 1992), the appearance and frequency of this new allele are consistent with the notion that the position 86 Gly/Val dimorphism is functionally important and subject to balancing selection. Recent structural analysis of the DR1 peptide binding groove indicates that, unlike class I peptide binding, the bound peptides extend through the ends of the groove (Brown et al. 1993) and suggests that the residues 57 and 86 at either end of the alpha-helix may be involved in the specificity and stability of peptide binding (Verreck et al.

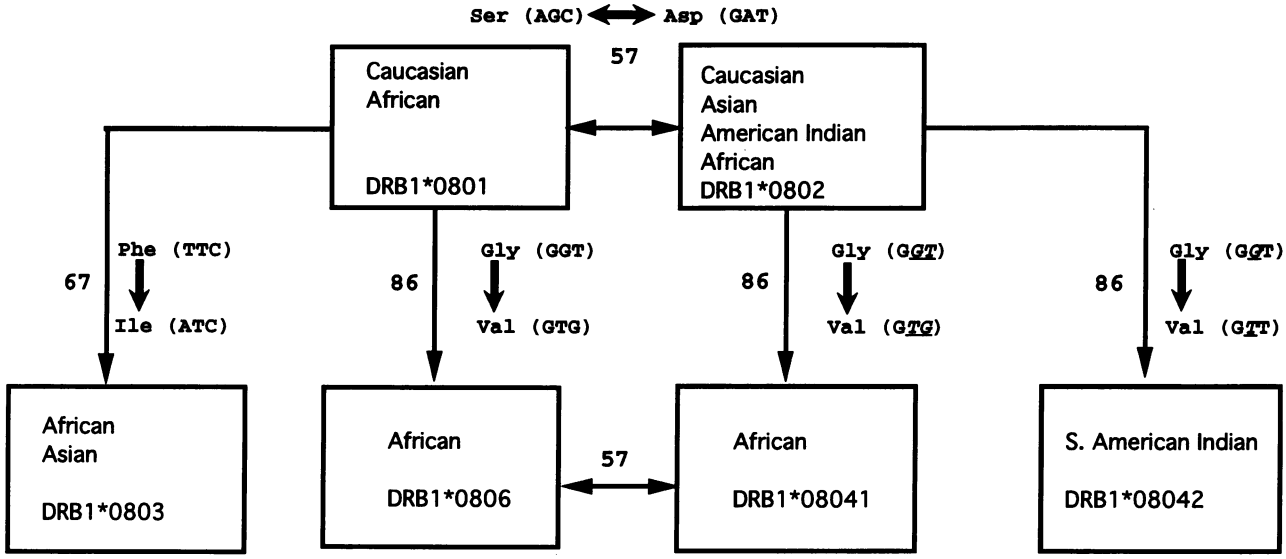


Figure 2 Distribution of DR8 alleles in different ethnic groups. Numbers associated with arrows indicate the codon positions where putative mutation or recombination events have generated the variability observed. DRB1*0801 and DRB1*0802 are assumed to be the ancestral alleles, because they are found in many different populations, and therefore are presumed to have been present prior to the separation of the races. The ethnically restricted DRB1 alleles presumably evolved from these cosmopolitan and ancestral alleles.

1993). We suggest that this allele, absent from all other populations, including all Amerindians studied to date, arose relatively recently and has subsequently been maintained, along with DRB1*0802, among the Cayapa. This novel allele brings the number of pairs of DRB1 alleles that differ only at position 86 to 10. Recently, an oligonucleotide probe analysis of South American Amerindians revealed a potentially new 86-variant of DRB1*0411 (Cerna et al. 1993). Moreover, we have recently identified another new DRB1*08 allele, DRB1*0807, in the Ticuna Indians of Brazil, which presumably arose from DRB1*0802 by a substitution in codon 57 (S. Mack and H. A. Erlich, personal communication). Although genetic drift cannot be formally excluded, the maintenance, in many different populations, of DRB1 allele pairs that differ only by Gly-86→Val-86 suggests the operation of balancing selection for position 86 variants.

Unlike all other DRB1 alleles, however, DRB1*08042 contains a GTT Val codon at position 86. All other DRB1 position 86 codons are either GGT (Gly) or GTG (Val), indicating that the Gly/Val substitutions that appear to have occurred relatively frequently in the evolution of DRB1 variants in human populations result from a complex mutation (GT↔TG) or a gene conversion/recombination event. In the evolution of DR8 diversity, the DRB1 allele *0804 appears to have been generated twice by independent mechanisms, in Africa by a GT→TG change and in South America by a G→T transversion. This convergent evolution to Val-86 variants of DRB1*0802, namely, DRB1*08041 and DRB1*08042, reflects the power of selective forces to maintain DRB1 diversity.

This view, which is based on the distribution of DR8 alleles in various human populations (illustrated in fig. 2), of recent (postseparation of human races) diversification of DRB1 alleles differs from the picture of DR8 diversity based on maximum-parsimony phylogenetic analysis, in the work of Klein et al. (1991). These authors infer that "the extant HLA-DRB1*08 polymorphism must have been generated in the last 6 myr [million years] or so" (p. 280). However, our interpretation of the sequence and population data (fig. 2) indicates that many of the DRB1*08 alleles arose *after* the separation of the major human races (~100,000 years ago) (Nei and Ohta 1991) and that some (e.g., DRB1*08042) may have been generated more recently, since the colonization of South America (10,000–20,000 years ago). In general, the evolution of DRB1-locus allelic diversity reflects sequence exchanges between ancient and conserved sequences within exon 2 β-sheet domains, with, however, more recent generation of α-helical domain variants by recombination or point mutations (Erlich and Gyllensten 1991), such as we see in the position 86 allelic variant DRB1*08042. The DRB1 allelic lineages (e.g., DR4 or DR8) appear to have arisen before hominoid speciation, with representative alleles found in several species; however, specific DRB1 alleles appear to have been generated postspeciation or, as in the case of some of the position 86 variants, after the separation of the human races.

In our view, the operation of balancing selection and convergent evolution at position 86 makes the phylogenetic analysis of a sequence data set such as the DR8 family, in which many of the alleles differ only at this codon,

extremely problematic. Position 86, like position 57, is a site of high homoplasy (frequent phylogenetically inferred changes) in phylogenetic trees (Erlich and Gyllensten 1989, 1991). The results reported here, like the recent analysis of new HLA-B variants in other South American Amerindian tribes (Belich et al. 1992; Watkins et al. 1992), are consistent with the notion of recent HLA diversification. The possibility that these HLA-B variants and DRB1*08042 were present in the human population prior to the migration to the Americas and then were lost everywhere but South America cannot be formally excluded but is less likely. Unlike the HLA-B variants, which appear to have been generated by recombinational mechanisms, DRB1*08042 arose from a point mutation. The appearance of this DRB1 allele emphasizes the central role of selection, in particular at position 86, in establishing HLA diversity, independent of the genetic mechanisms of sequence diversification.

The origin of the DRB1*08042 (Val-86) allele from DRB1*0802 (Gly-86) in the Cayapa, like the appearance of DRB1*08041 (Val-86) from DRB1*0802 in Africans (fig. 2) (Apple and Erlich 1992), reflects positive selection for new DRB1 position 86 variants in these two populations. The time of origin for the African DRB1 allele, generated by a GT→TG change, cannot be estimated easily. However, the new Cayapa allele, which resulted from a T→G substitution, presumably arose after the separation of the Cayapa from North Amerindians and other South Amerindians. The presence of this recent DRB1 allele, *08042, on three different DR/DQ haplotypes suggests positive selection among the Cayapa for new DR/DQ combinations generated by recombination, between DQA1 and DQB1, or, in the case of other haplotypes, between DQA1 and DRB1. The general diversity of DR/DQ haplotypes (table 2) seen in this small and isolated population is consistent with such selection pressures.

In the Cayapa, the unusually high frequency (50%) of DPB1*1401, an allele rare or absent in all other Amerindian groups studied to date as well as in other human populations, may reflect genetic drift or, more likely, positive directional selection, such as resistance to infectious disease pathogens. An alternative hypothesis to the high frequency of DPB1*1401 in the Cayapa could be an Asian/Polynesian-Pacific origin; however this theory was not supported by class II data from a Hawaiian population (E. Titus-Trachtenberg, unpublished observations). Our analysis of class II loci in the Cayapa, taken together with the recent studies of HLA-B loci in other Amerindian groups (Belich et al. 1992; Watkins et al. 1992), suggests that newly arisen HLA variants, generated either by point mutation, gene conversion, or recombination between loci, have been selected for and maintained, along with other alleles and haplotypes, in these South Amerindian populations.

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