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Further diversification of the *HLA-B* locus in Central American Amerindians: new *B*39* and *B*51* alleles in the Kuna of Panama

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Several new *HLA-B* locus alleles have been discovered in South American Amerindians. By contrast, analysis of the MHC class I alleles of North American native populations has revealed few new *HLA-B* alleles. This suggests that the *HLA-B* locus is evolving rapidly in South American populations. Here we describe the *HLA-B* locus alleles present in individuals from a Central American tribe, the Kuna of Panama. Using a sequence-based typing technique that separates alleles by denaturing gradient gel electrophoresis (DGGE) followed by direct sequencing, we determined the *HLA-B* alleles from eight Kunas. Two of the *HLA-B* alleles present in the Kuna have been previously described in other South American Amerindian populations; one allele has been characterized in a Mexican-American. We characterized two new *HLA-B* alleles in the Kuna, *HLA-B*3911* and *HLA-B*5110* . *HLA-B*3911* differed from *HLA-B*3905* by only a single nucleotide substitution in exon 3. This substitution resulted in an amino acid replacement of leucine by arginine at residue 156 in the alpha 2 domain. Such a change may affect the repertoire of peptides that are bound by this molecule. *HLA-B*5110* differed significantly from other *HLA-B*51* alleles in that it is the result of an unusually large intra-locus recombination event of minimally 216 nucleotides. This recombination results in an allele that is part *HLA-B*51* and part *HLA-B*40*. Thus, more dramatic recombination events may also play a role in the rapid evolution of the *HLA-B* locus in Amerindians.

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Of the highly polymorphic major histocompatibility complex (MHC) class I genes, the *B* locus appears to be evolving more rapidly than any other MHC class I locus. Seventeen new *HLA-B* alleles have been recently reported from seven South American Amerindian tribes (1–6). Molecular sequence analysis of chimpanzees and bonobos indicates that the *B* locus is characterized by intra-locus recombination (7). The rapid evolution of the *HLA-B* locus in the Amerindian populations from South America, however, contrasts with the limited number of new variant *HLA-B* alleles found in North American tribes (1, 8, 9).

Despite extensive analysis of *HLA-B* alleles in North and South American Amerindians, the *HLA-B* locus alleles of Central American Amerindians remain largely uncharacterized. Six new *HLA-B* alleles from Mexicans and individuals of Mexican descent (10–15) have been described, hinting at the allelic diversity in these populations. Here we report the molecular *HLA-B* typings for eight individuals from a group of Central Amer-

The names *HLA-B*3911* and *HLA-B*5110* have been officially assigned by the WHO Nomenclature Committee in October 1996 and May 1997. This follows the agreed policy that, subject to the conditions stated in the most recent nomenclature report (32), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO nomenclature report. The nucleotide sequences of *HLA-B*3911* and *HLA-B*5110* have been deposited in GenBank and assigned the accession numbers U74368 and AF004370, respectively.

ican Amerindians, the Kuna of Panama. The sequences of these *HLA-B* alleles were determined by denaturing gradient gel electrophoresis (DGGE) followed by direct sequencing as described by Eberle et al. (16).

Material and methods

Cell culture and RNA isolation

Blood samples were collected from eight Kuna individuals. The Kuna belong to the Paya-Chibchan linguistic phylum and currently number 65,000. The Kuna presently inhabit islands along the San Blas Archipelago with much smaller populations around Lake Bayano and Paya, next to the Colombian border. Hypotheses regarding the origins and history of the Kuna remain controversial (17).

Peripheral blood lymphocytes were isolated from whole blood by density gradient centrifugation on Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ, USA). B-lymphoblastoid cell lines were generated by incubating B cells with supernatants from the Epstein-Barr virus-producing B-958 cell line. Total RNA was extracted from either peripheral blood lymphocytes and B-lymphoblastoid cell lines using RNAzol-B (Tel-Test, Friendswood, TX, USA) following the manufacturer's instructions.

DGGE and direct sequencing of Kuna *HLA-B* alleles

HLA-B locus alleles were analyzed using DGGE followed by direct sequencing as described by Eberle et al. (16). Briefly, total RNA was reverse transcribed and PCR amplified (RT-PCR) using both a one step RT-PCR kit (RNA Access RT-PCR Kit, Promega, Madison, WI, USA) and a two-step method. Amplification primers were A1MID, which anneals to the middle of exon 2, and the GC-tailed primer, B-A3MID+GC, an *HLA-B* locus-specific primer that anneals to the middle of exon 4. GC-clamped amplification products were separated using DGGE, which separates identically sized DNA fragments based on sequence composition (18) over a urea and formamide gradient. DGGE-separated RT-PCR products were reamplified with universal sequencing tailed primers. The tailed PCR products were directly sequenced using ABI Prism Dye Primer Cycle Sequencing Core Kit with AmpliTaq DNA Polymerase, FS and the −21M13 and reverse M13 dye primers (Perkin Elmer-Applied Biosystems, Foster City, CA, USA). Sequencing reactions were loaded onto a 373 DNA Sequencer (Perkin Elmer-Applied Biosystems). Derived sequences were aligned to a database of known *HLA-B* alleles using FACTURA HLA (Perkin Elmer-Applied Biosystems).

Table 1.
HLA-B alleles from eight Kuna Amerindians

Sample	*B1*	*B2*
Kuna 10	*B*3501*[a]	*B*3512*[b]
Kuna 13	*B*3903*[c]	*B*3911*[d]
Kuna 14	*B*1522*[e]	*B*5110*[d]
Kuna 15	*B*3503*[a]	–[f]
Kuna 16	*B*1522*[e]	*B*3501*[a]
Kuna 17	*B*1522*[e]	*B*3903*[c]
Kuna 20	*B*3903*[c]	*B*3911*[d]
Kuna 21	*B*3501*[a]	*B*4002*[a]

[a] Alleles found in Caucasians and Asians (19–22).
[b] Allele found in Mexican-Americans (11).
[c] Allele found in the Waorani of South America (1).
[d] Allele unique to the Kuna of Central America.
[e] Allele found in the Cayapa of South America (3).
[f] Dash indicates our inability to isolate a second *B* locus allele and thus, this individual is likely homozygous at the *HLA-B* locus.

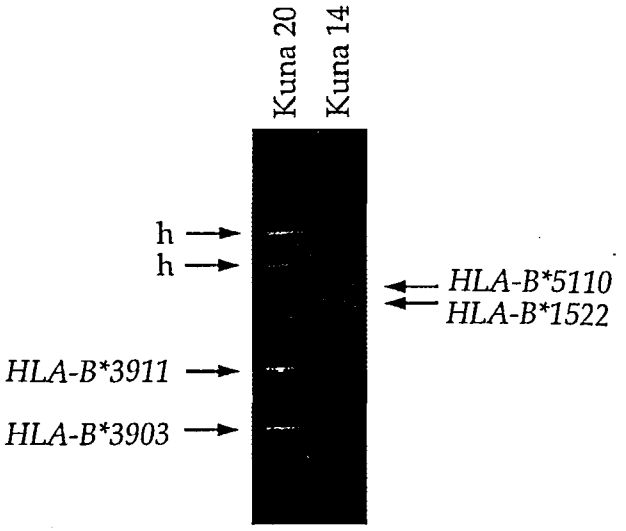

Figure 1. Separation of *HLA-B*3911* and *HLA-B*5110* from Kuna 20 and 14 on a 40–60% parallel DGGE gel. *HLA-B* locus alleles were analyzed using DGGE followed by direct sequencing (16). The other alleles in Kuna 20 and 14 are indicated: *B*3903* in Kuna 20 and *B*1522* in Kuna 14. The small letter h designates heteroduplex bands formed between *HLA-B*3903* and *HLA-B*3911* during RT-PCR as described by Eberle et al. (16).

Cloning and sequencing

To confirm the *HLA-B*3911* and *HLA-B*5110* sequences, the amplification products were cloned and sequenced from Kuna 20 and Kuna 14, respectively. For both new alleles, RT-PCR was repeated under the same conditions using B-A3MID (without the GC-clamp) and an alternative 5′ primer, A1START+M13, which anneals to the beginning of exon 2. This 5′ primer anneals outside A1MID, thus providing additional sequence information. RT-PCR products were resolved on 1% agarose,

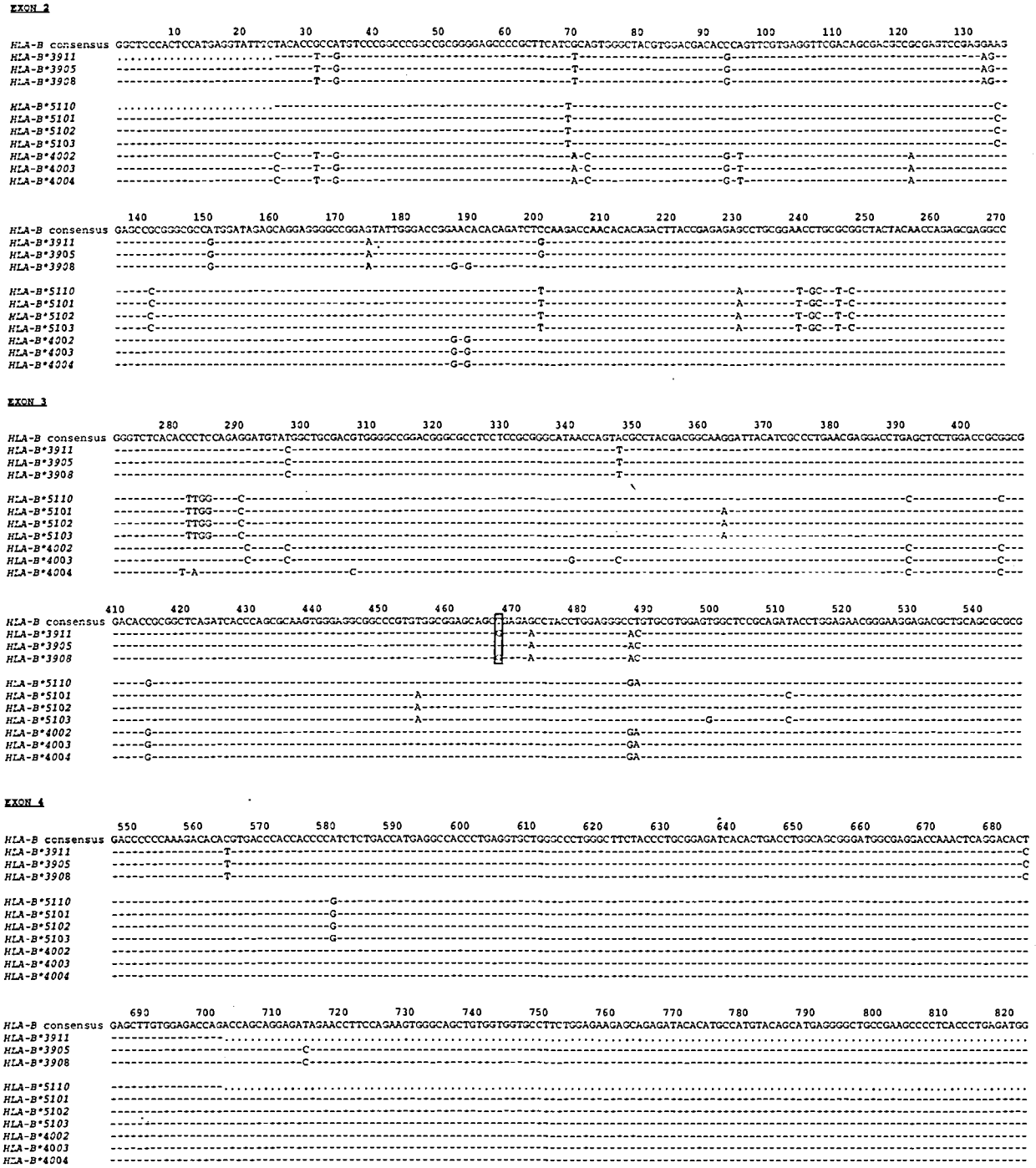

Figure 2. Nucleotide sequences of *HLA-B*3911* and *HLA-B*5110* aligned to an *HLA-B* consensus (23). Exons 2, 3, and 4 are indicated. Sequences of *HLA-B*3905* and *HLA-B*3908* (11) are included as the two possible recipient and donor alleles, respectively, involved in an intra-locus conversion event resulting in *HLA-B*3911*. The single nucleotide substitution in *HLA-B*3911* is boxed. The sequences of *HLA-B*5101*, *HLA-B*5102*, and *HLA-B*5103* are aligned as possible recipient alleles involved in the recombination event that generated *HLA-B*5110* (23, 24). *HLA-B*4002*, *HLA-B*4003*, and *HLA-B*4004* are possible donor alleles found in the contemporary pool of Amerindian *HLA-B* alleles (23, 24). Dashes (−) denote sequence identity to the consensus. Periods (.) indicate gaps introduced to maximize the alignment.

and the appropriate band was excised. Amplification products were purified from the agarose gel using an anion-exchange resin (Qiagen, Chatsworth, CA, USA) and were cloned into the TA cloning vector, pCR2.1 (Invitrogen, San Diego, CA, USA). Ligation mixtures were used to transform One-Shot competent *Escherichia coli* cells (Invitrogen). Multiple clones were isolated and se-

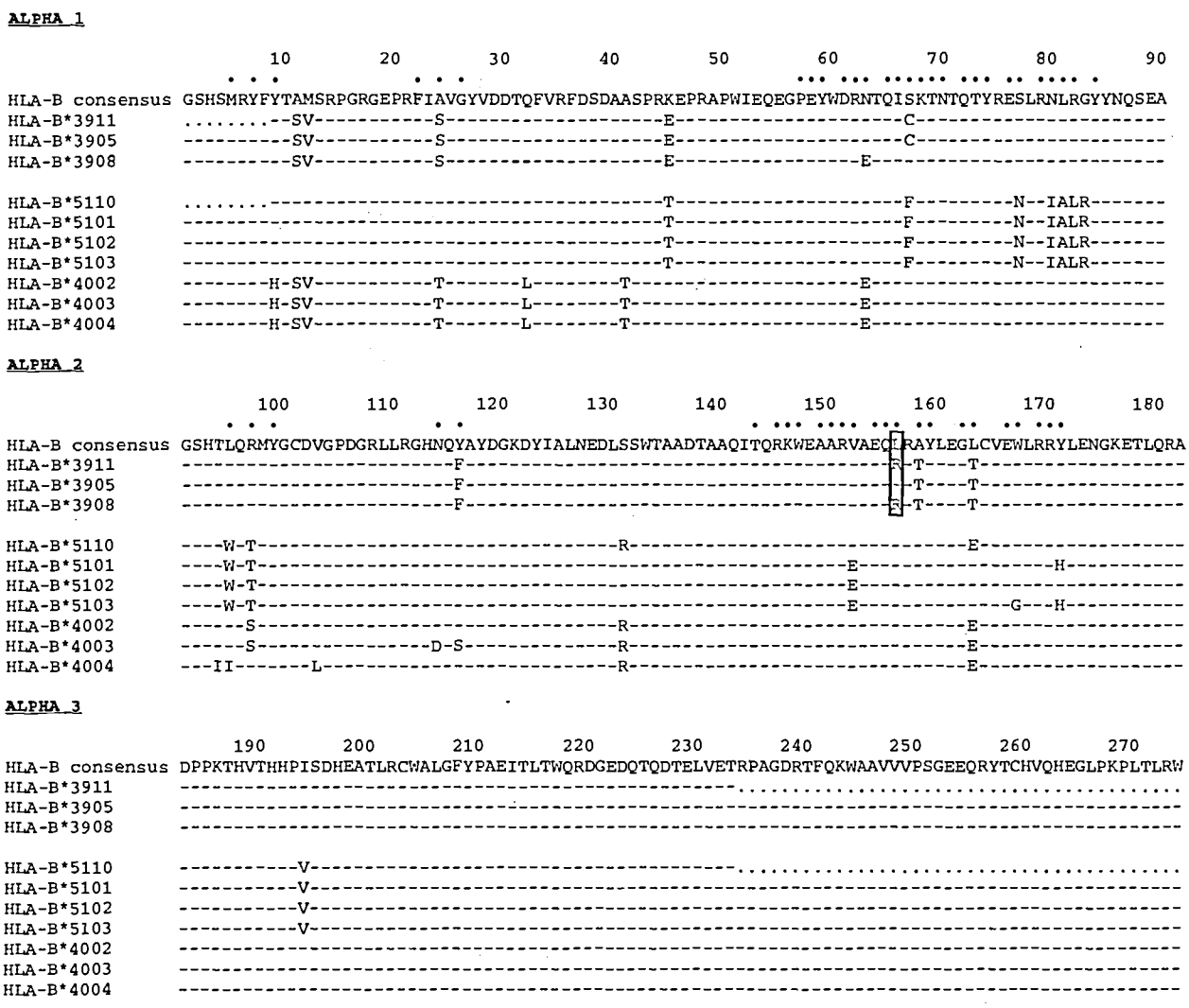

Figure 3. Predicted amino acid sequences of HLA-B*3911 and HLA-B*5110 compared to an HLA-B consensus (23). The alpha 1, 2 and 3 regions are indicated. The leucine to arginine replacement at residue 156 in HLA-B*3911 is boxed. Dashes (–) indicate identity with the HLA-B consensus, bullets (●) denote residues that line the peptide-binding region (25), and periods (.) represent gaps introduced to maximize the alignment.

quenced using fluorescent dye-labeled dideoxy terminators (Perkin-Elmer–Applied Biosystems). Sequencing reactions were run on an ABI 373 automated sequencer. Both strands of three clones were sequenced to reduce the possibility of reporting PCR-generated artifacts.

Results

To investigate whether new HLA class I alleles were present in Central American Amerindians, we molecularly typed the *HLA-B* loci of eight Kunas from Panama. After separation by DGGE, these alleles were reamplified and directly sequenced. Analysis revealed that like South American Amerindians, the Kuna lymphocytes expressed a limited number of *HLA-B* alleles: *HLA-B*1522*, *B*3501*, *B*3503*, *B*3512*, *B*3903*, *B*3911*, and *B*4002* (Table 1). Two of these alleles, *HLA-B*1522* and *HLA-B*3903*, were originally described in Amerindians of two different Ecuadorian groups: *B*1522* was found in the Cayapa (3) and *B*3903* in the Waorani (1). Additionally, *HLA-B*3512* was originally discovered in an individual of Mexican-American descent (11).

Interestingly, we identified two new *HLA-B* alleles in the Kuna, *HLA-B*3911* and *HLA-B*5110* (Figure 1). *HLA-B*3911*, found in both Kuna 13 and 20, was most similar to *HLA-B*3905*, differing by only a single nucleotide at position 467 in exon 3 (Figure 2). This mutation in *HLA-B*3911* changed the corresponding codon in *HLA-B*3905* from CTG to CGG, resulting in an amino acid replacement at position 156 from leucine to argi-

nine (Figure 3). In *HLA-B*5110* from Kuna 14, exon 2 encoding the alpha 1 domain was identical to either *HLA-B*5101*, *B*5102*, or *B*5103* while exon 3 encoding the alpha 2 domain was most similar to either *HLA-B*4002*, *B*4003*, or *B*4004*. However, the alpha 3 domain encoded by exon 4 was identical to *HLA-B*5101*, *B*5102* or *B*5103* (Figures 2, 3).

Sequence analysis of cloned cDNAs from Kuna 20 confirmed that *HLA-B*3911* differed from *HLA-B*3905* only at nucleotide 467 of exon 3 (Figure 2), resulting in the amino acid replacement at position 156 (Figure 3). Analysis of cloned cDNAs from Kuna 14 also confirmed the unique nucleotide sequence of *HLA-B*5110*: exons 2 and 4 are identical to *HLA-B*5101*, *B*5102*, or *B*5103*, but exon 3 is most similar to *HLA-B*4002*, *B*4003*, or *B*4004* (Figure 2).

Discussion

The limited characterization of *HLA-B* alleles in Central American Amerindians contrasts with the extensive analyses of *HLA-B* alleles in North and South American Native Americans (1–6, 8, 9). To investigate whether the *HLA-B* locus is evolving rapidly in Central American Amerindians, we undertook molecular characterization of the *HLA-B* locus from eight Kuna individuals using a sequence-based typing technique of DGGE followed by direct sequencing (16). Like previously described South American Amerindians, the Kuna expressed only a small number of *HLA-B* alleles. Interestingly, in the course of our analysis we described two new *HLA-B* variants, *HLA-B*3911* and *HLA-B*5110*.

*HLA-B*3911* and *HLA-B*3905* differed by only one nucleotide at position 467 in exon 3 (Figure 2). This one difference resulted in residue 156 of the alpha 2 domain changing from leucine to arginine (Figure 3). This replacement of a small, nonpolar amino acid with a large, very basic amino acid could affect the pool of peptides bound by this molecule. Amino acid 156 in the alpha 2 domain is considered a polymorphic site and lines the peptide-binding region (25). More specifically, residue 156 lines the D pocket of the peptide binding region, a region that is usually hydrophobic (26).

No *HLA-B* alleles so far detected in South American Amerindians (as reviewed by Parham & Ohta; 24) possess arginine at residue 156. However, *HLA-B*3908*, originally described in a Mexican-American (11), possesses arginine at position 156 that is encoded by the same substitution at nucleotide 467. This suggests that *HLA-B*3911* could have been generated through intra-locus conversion involving the *HLA-B*3908* donor allele and a recipient *HLA-B* allele present in the founding population or in the contemporary pool of South American Amerindian alleles. Of the contemporary alleles, *HLA-B*3905* has greatest sequence similarity to *HLA-B*3911*. Thus, this allele is a likely candidate for intra-locus conversion with *HLA-B*3908*. Alternatively, the *HLA-B*3911* allele could have arisen by point mutation of nucleotide 467 of *HLA-B*3905*.

The second new allele found in the Kuna, *HLA-B*5110*, is the product of an unusual recombination event (Figure 2). Rather than single or clustered nucleotide differences that can be attributed to either point mutation or small segmental exchange, *HLA-B*5110* is the product of a much larger recombination between an *HLA-B*51* allele (*HLA-B*5101*, *B*5102* or *B*5103*; Figure 2) and an *HLA-B*40* allele (*HLA-B*4002*, *B*4003* or *B*4004*; Figure 2). Exchange of nucleotides between *HLA-B* alleles in South American Amerindian populations appears to be a fairly common event and is usually the result of small segmental exchange resulting in the exchange of 1 to 27 nucleotides (1–6). Only one allele of the previously described new alleles, *HLA-B*4802*, has a large recombination of 198 nucleotides (1). Interestingly, *HLA-B*5110* shows minimally 216 nucleotides exchanged between an *HLA-B*51* recipient allele (*HLA-B*5101*, *B*5102* or *B*5103*; Figure 2) and an *HLA-B*40* donor allele (*HLA-B*4002*, *B*4003* or *B*4004*; Figure 2). The nucleotide sequence of *HLA-B*5110* is identical to *HLA-B*5101-03* except for nucleotides 363–579 of exon 3, where it is identical to *HLA-B*4002-04*. Additionally, *HLA-B*1520*, described in the OLGA South American Amerindian reference cell line of the 10th International Histocompatibility Workshop, is the product of a large recombination event where exons 1 and 2 are identical to *HLA-B*1501* while exons 3–7 and the 3′ untranslated region are identical to *HLA-B*3501* (27). Such data suggest that recombination of large segments of DNA may be a rare event in the generation of new *HLA-B* alleles in Amerindian populations but that intra-locus conversion of smaller segments of DNA may have a much larger role. Recent examination of intron 2 sequences suggests that intronic intra-locus conversion in addition to exonic conversion may be a mechanism for generation of new *HLA-B* alleles in Amerindians (28).

Most of the newly discovered *HLA-B* alleles in South America differ from their previously described counterparts by a few nucleotide substitutions in exon 3 encoding the alpha 2 domain. It is unlikely that these small changes in the alpha 2 domain cause profound differences in peptide

binding (29). The alpha 1 domain of HLA-B*5110 is identical to several HLA-B*51 molecules (HLA-B*5101, B*5102 or B*5103; Figure 3), whereas the alpha 2 domain is most identical to several HLA-B*40 molecules (HLA-B*4002, B*4003 or B*4004; Figure 3). How this structural feature of HLA-B*5110 might affect the peptides bound by this molecule and whether such an unusual molecule will bind peptides similar to already characterized peptide motifs in other HLA-B51 molecules remains to be seen (30). Barber et al. (31) characterized the peptide motifs for HLA-B*4601, an interlocus recombinant between HLA-B*1501 and HLA-Cw*0102 where residues 66–76 of the alpha 1 domain are from HLA-Cw*0102. They found that this molecule behaves like the HLA-C parental allotype in its peptide binding motifs and function (31). It is possible, therefore, that the new HLA-B*5110 molecule might bind peptides with sequences similar to those bound by HLA-B51.

The description of *HLA-B*3911* and *HLA-B*5110* in the Kuna of Panama supports hypotheses regarding the evolution of HLA alleles in Amerindian populations (1–3, 9). Additionally, since both HLA-B*3911 and HLA-B*5110 differed from their previously described counterparts in the peptide-binding region, positive selective pressure may have maintained these new alleles in the Kuna population. During the migration of the founding Amerindian population across the Bering Land Bridge and through North America to Central America, the population could have encountered new pathogens. These agents may have exerted positive selective pressure on the *HLA-B* alleles of the migrating Amerindians.

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