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HLA genes in Cubans and the detection of Amerindian alleles

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Abstract

Caribbean Islands including Cuba were first inhabited by Meso-American and later by Arawak-speaking Amerindians from nowadays Venezuela. Spanish invaders brought to almost extinction to the Amerindian population after 1492. Black slaves from West Africa were taken into Cuba by Europeans. The degree of admixture among populations is approached. HLA alleles were studied by DNA techniques. Comparison with other worldwide populations (a total of 14.094 chromosomes) included genetic distances, Neighbour-Joining dendrograms, correspondence analyses and calculation of extended haplotypes. While African-European HLA features were clearly found, Amerindian HLA characteristics are less evident, indicating that Amerindian devastation was particularly marked after 1492 AD. However, typical Amerindian alleles have been found in our Cuban sample, i.e. DRB1*0403, DRB1*0404, DRB1*0407, BRB1*0411, DRB1*0802 and DRB1*0809. The presence of Amerindian alleles in Cubans may have a bear in the making up of transplantation registries (both for bone marrow and solid organ transplantation) at the regional level and also be important for epidemiological studies of diseases linked to HLA.

Keywords: Africans; Amerindians; Arawak; Ciboneys; Cuban; HLA; Tainos

1. Introduction

Cuba was closer to Florida Keys and Bahamas Islands at the beginning of the Holocene Epoch (Moure and de la Calle, 1996) because sea level was lower (see Fig. 1). However, although an immigration from Mississippi culture people crossing through Florida is possible, no archaeological data support it (Wilson, 1997). In fact, the first archaeological sites in Cuba started earlier than 4000 years BC and were brought by Yucatan Peninsula people, Meso-Americans, not excluding any route, including Florida. They were hunter-gatherers (Wilson, 1997). Archaeological variants from this culture are found in Cuba by 2000 BC. At the same time also non-agricultural people coming from North South America invaded the Caribbean, including Cuba (Wilson, 1997). These at least two kind of people lived for long in the Caribbean Islands until about 500 years BC, when people coming again from North South America (Arawaks from Venezuela) arrived with ceramic and agriculture technology and spread thoughout the Islands (Wilson, 1997). When Columbus arrived (1492 AD) many complex chiefdoms were found in Cuba and other Islands (Wilson, 1997).

Cuba had two different kind of Amerindians at the time of Spaniard invasion in 1492 AD as described by father Bartolome de las Casas: pre-agricultural Ciboney and agricultural Taino people (Moure and de la Calle, 1996). The latter being Arawak language-speakers. Other anthropophagic-warrior Amerindians were living in the small Antillean Islands and were considered as a threat by the other Caribbean Amerindians (Moure and de la Calle, 1996). Pre-agricultural Ciboney Cubans mostly lived in the westernmost part of the Island. Soon, Cuban Amerindians were disappearing by slavery conditions, war (against Spaniards) and Spaniards-born diseases (mainly, small-pox,

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Fig. 1. Map of Cuba Island and Caribbean surroundings. Prehistorical and historical immigrations and their timing are indicated.

measles, influenza and yellow fever). Later, Spaniards carried massively Black Africans slaves into the Islands (Wilson, 1997) (see Fig. 1).

In summary, nowadays genes in Cuba must come from Europeans, Africans and also Amerindian gene traces should be noticed, even if Amerindians were exterminated after 1492 AD. In the present work, we aim to uncover the presence of genes corresponding to the proposed invasions by using HLA genotyping; this methodology distinguishes quite accurately between Amerindian and non-Amerindian genes and more difficulty between Mediterranean and African genes.

2. Materials and methods

2.1. Population sample

Seventy-eight unrelated healthy voluntary blood donors were studied. They were chosen from BETERA Laboratories (Marianao Blood Bank Center, Havana, Cuba) cellular panel; regardless racial, sex or any other differences among individuals, but we did know by inquiry and direct observation, for further data analysis, donors anthropometric phenotypes, putatively classified as: Caucasoid-like (67%), Mixed [Mestizos/mulattos (21%)] or Blacks (11%). Havana is the best place to sample a Cuban population since it has been the main immigration center from all parts of the Island.

2.2. DNA extraction

Genomic DNA from peripheral blood mononuclear cells was extracted with a Nucleic Acid Extractor. (Applied BioSystem, Foster City, California) with the use of ABI reagents and protocols (Martinez-Laso et al., 2001) or using the salting-out method (Miller et al., 1988).

2.3. HLA-DNA typing

HLA class I (A, B) and HLA class II (DRB1, DQA1 and DQB1) typing were performed using a reverse dot-blot technique with the Automated Innolipa system (Innogenetics N.V., Zwijndrecht, Belgium). HLA-A, -B, -DRB1, -DQA1 and -DQB1 allele DNA sequencing was only done in an automated Applied Biosystems DNA sequencer, when this indirect DNA typing yielded ambiguous results (Arnaiz-Villena et al., 1992); direct sequencing for both class I and class II alleles were used to confirm those alleles found to be relevant for some haplotypes combinations and in cases of non-deducible oligotyping due to oligonucleotide probes failures. Sequencing reactions were carried out using Sanger's dideoxy chain termination method on an Applied Biosystems automated DNA sequencer as previously described (Gocayne et al., 1987).

2.4. Statistical analysis

Statistical analysis was performed with Arlequin v.2.000 software kindly provided by Schneider et al. (2000). In summary, this program calculated HLA-A, -B, -DRB1 and -DQB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between two alleles at two different loci. Their level of significance (p) for 2×2 comparisons was determined as previously described (Imanishi et al., 1992a,b). In addition, the frequency of maximum likelihood complete haplotypes were deduced from: (1) the 2, 3 and 4 HLA loci haplotype frequencies (Imanishi et al., 1992a,b); (2) the previously described haplotypes in other populations (Imanishi et al., 1992a,b); and (3) haplotypes if they appeared in two or more individuals and the alternative haplotype was well defined (Imanishi et al., 1992a,b). In order to compare phenotype and haplotype HLA frequencies with other populations, the reference tables of the 11th and 12th International HLA Workshops were used [(Clayton and Lonjou,