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Genetic Structure of Cartagena de Indias Population Using Hypervariable Markers of Y Chromosome

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Abstract

Ethnicity has been associated with the incidence of diseases and consequently it is a cornerstone in medical genetic studies. It is mainly important in admixture populations, where the population stratification can produce spurious results that lead to erroneous conclusions. Consequently, population stratification has become one of the most important confounding factors in population-based genetic association studies, especially in Latino populations. Cartagena de Indias is a cosmopolitan city with dissimilar ancestry proportions due to recent miscegenation. This population mainly exhibits African and Amerindian matrilineal ancestries. Nevertheless, important asymmetries in the paternal genetic history related to the complex patterns of migration in the colonial period increase the male genetic diversity in this population. As a result of this recent admixture, population stratification has arisen, where each subpopulation is not equally represented. Consequently, the allele differences between cases and controls could be related with different frequencies among different population strata rather than the association of the genes with the disease. In order to define the patrilineal substructure of the Cartagena's population, a total of 130 unrelated men were ancestrally studied using 15 Y-STR loci routinely employed in anthropological, forensic and population genetics. Our results show that Cartagena is an admixture population consisting of European (80%), Amerindian (10%) and African ancestries (10%), which are

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represented by haplogroups R1b and I2a (xI2a1), Q-M242/Q-M3, and E1b1a/E1b1b, respectively. Complex genetic patterns found in Cartagena's population emphasize the importance to know the genetic variation in order to diminish the inconsistence for future genetic association studies. In addition, our findings illustrate the complex genetic background of Cartagena population and reinforce the need to encompass more geographic regions to generate more robust data for anthropological and forensic applications.

Keywords

Population Genetics, Y-Chromosome, Microsatellites, Cartagena, Colombia

1. Introduction

Cartagena de Indias is a city in the north of Colombia located on the shores of the Caribbean Sea and is the capital of the Department of Bolivar. Cartagena is one of the oldest cities on the Colombian Caribbean coast founded in 1533 by Spanish conquerors [1]. This city also represented the primary slaving port during the early colonial period (1570-1640), where more than 150,000 Africans coming from western Africa (Angola and the Guineas, principally) were introduced into Americas [2]. As a consequence, the ethnic composition of Cartagena is the result of a three-hybrid fusion: the native aborigines known as the Calamari; the European conquerors (principally from Spain); and the African slaves [3]. This ancestral admixture has predominantly resulted in a mulatto, mestizo, Amerindian, and Afro-descendant population. However, recent evidences show a disproportionate contribution of European males and Amerindian females, provoking a sex bias in admixture proportions [4] [5].

Genetic association studies are a powerful strategy for identifying genotypic-phenotypic associations in complex diseases. Recent findings confirm that populations with multiple ethnic origins show important differences in allelic and genotypic frequencies, which may inflate false positive rates causing a spurious association because the genetic stratification is unevenly distributed across different subpopulations [6]. Therefore, allele differences between cases and controls could be related with systematic differences in ancestry as well as dissimilar frequencies among different population strata rather than a real association of the genes with the disease [7] [8]. These differences are more important in young populations with complex patterns of admixture such as Latin American populations [9]. Hence, population stratification is the principal confounder variable on genetic association studies, causing bias that may yield misleading results, which could be used in the practice of medicine and public health [10].

Focused on identifying the patrilineal contribution in Cartagena's population and to avoid spurious associations, we determined the genetic structure of Cartagena de Indias population using Y-Chromosome Short Tandem Repeats (Y-STR). We analyzed 130 unrelated men, using 15 Y-STR loci which is routinely employed in human migration and evolutionary studies as well as genetic structure and forensic analysis among others [11] [12]. Our findings show that Cartagena's population is highly diverse, showing patrilineal ancestry from European, Middle Eastern, African and Amerindian populations. These findings highlight the importance of knowing local-specific patterns throughout the country in order to establish the population stratification and correct the impact of admixture in future genetic association studies. In addition, genetic variability studies provide information about population history, as well as the relationship between some diseases and ancestral lineages, which could also be used in the subsequent epidemiological studies [13].

2. Materials and Methods

2.1. Population

We analyzed the STR genetic data of 130 unrelated individuals born in Cartagena de Indias, Colombia with at least three generations of ancestors who had been born in this city (**Figure 1**). The questionnaire for choosing candidates was designed to ensure that the participants were not filially related, *i.e.*, no one was son or brother of another. This study was approved by the Ethics Research Committee of the University of Cartagena, Colombia (Resolution Number 46, 2012). Each individual signed an informed consent to participate in this study.

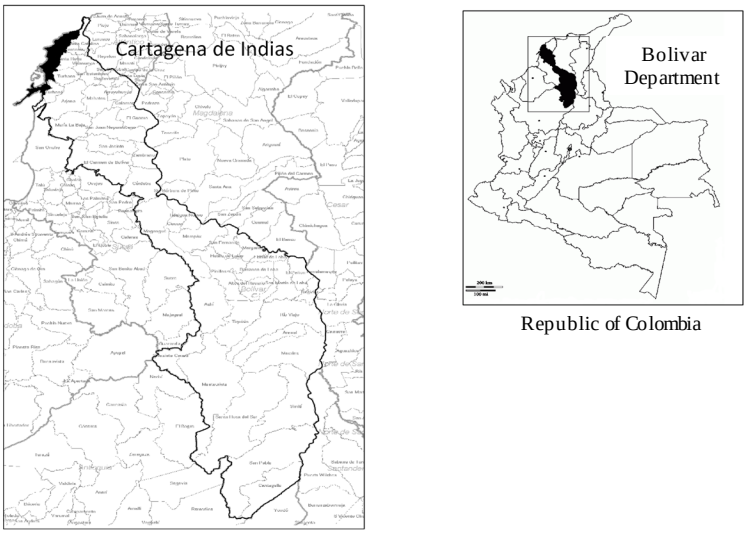

Figure 1. Map of the republic of Colombia showing Bolivar Department and the location of cartagena de Indias.

2.2. Molecular Analysis

Genomic DNA was extracted from peripheral blood leukocytes using Qiamp DNA mini kit (Qiagen, Düsseldorf, Germany). Approximately 10 ng target DNA was amplified using fifteen Y-chromosome short tandem repeat markers described previously (**Table 1**). Amplicons were obtained using multiplex reactions described in **Table 2**. The resulting amplicons were carried out on the ABI Prism 3130XL Genetic Analyzer using GeneMapper ID v.3.2. software (Applied Biosystems, Carlsbad, CA, USA).

2.3. Quality Control

Control DNA 007 was used as international validated internal controls (Applied Biosystems, Carlsbad, CA, USA).

2.4. Statistical Analysis

Allelic and haplotypic frequencies, number of alleles (*k*), haplotype diversity (HD), genetic diversity over loci (*h*), and mean pairwise differences (M) were estimated using Arlequin software v 3.5 [14]. The number of unique haplotypes (UH) was estimated by direct counting. In order to compare our data with other populations, haplotype and haplogroup information was collected from previous reports. A total of 1372 individuals from different Colombian populations were included in the database and used for further analysis [15]-[17]. Y haplogroups were determined from Y-STR haplotypes with haplogroup predictor software (http://www.hprg.com/hapest5/), using equal priority to estimate the probability of assignment to a particular haplogroup [18]. The phylogenetic relationship of STR haplotypes was analyzed with Network v 4.6.1.1 software [19], which was built with Network Publisher software using a median joining approach and MP post-processing. Each haplotype was connected to all other haplotypes from which it differed by one repeat unit step at a single microsatellite locus. The Y-STR loci were weighted based on the inverse of their variances.

3. Results

3.1. Y-Chromosome STR Diversity

The haplotype distribution of the 15 Y-STR loci in the 130 individuals studied is shown in **Table 3**. The distribution of allele frequencies, number of different alleles (*k*), and locus diversity (*h*) are shown in **Table 4**. The most diverse loci were DYS458 ($h = 0.775$), DYS438/DYS390 ($h = 0.766$), DYS635/DYS389II ($h = 0.740$), and DYS19 ($h = 0.721$); while DYS393 ($h = 0.406$) was the least diverse.

Table 1. Primer sequences used for the multiplex reactions.

Uorochrome	STR Locus		Primer Sequence	Primer Seq. Ref.
PET	DYS19	F	ACTACTGAGTTTCTGTTATAGTGTTTTT	[11]
		R	GTCAATCTCTGCACCTGGAAAT	
VIC	DYS389I	F	CCAACTCTCATCTGTATTATCTATG	[11]
		R	GTTATCCCTGAGTAGTAGAAGAATG	
VIC	DYS389II	F	CCAACTCTCATCTGTATTATCTATG	[11]
		R	GTTATCCCTGAGTAGTAGAAGAATG	
VIC	DYS390	F	TATATTTTACACATTTTTGGGCCC	[40]
		R	GTGACAGTAAAATGAAAACATTGC	
6-FAM	DYS391	F	TTCAATCATACACCCATATCTGTC	[11]
		R	GATAGAGGGATAGGTAGGCAGGC	
NED	DYS392	F	TAGAGGCAGTCATCGCAGTG	[40]
		R	GACCTACCAATCCCATTCCTT	
VIC	DYS393	F	GTGGTCTTCTACTTGTGTCAATAC	[11]
		R	GAACTCAAGTCCAAAAAATGAGG	
6-FAM	DYS437	F	GACTATGGGCGTGAGTGCAT	[40]
		R	GAGACCCTGTCATTCACAGATGA	
VIC	DYS438	F	CCAAAATTAGTGGGGAATAGTTG	[11]
		R	GATCACCCAGGGTCTGGAGTT	
NED	DYS439	F	TCGAGTTGTTATGGTTTTAGGTCT	[11]
		R	GTGGCTTGGAATTCTTTTACCC	
PET	DYS448	F	TGGGAGAGGCAAGGATCCAA	[11]
		R	GTCATATTTCTGGCCGGTCTGG	
VIC	DYS456	F	GGACCTTGTGATAATGTAAGATAG	[41]
		R	GTAGAGGGACAGAACTAATGGAA	
6-FAM	DYS458	F	GCAACAGGAATGAAACTCCAAT	[11]
		R	GTTCTGGCATTACAAGCATGAG	
NED	DYS635	F	ACCAGCCCAAATATCCATCA	[42]
		R	TGGAATGCTCTCTTGGCTTC	
6-FAM	Y-GATA-H4	F	GAGACCTAAGCAGAGATGTTGGTTTTC	[41]
		R	CCTCTGATGGTGAAGTAATGGAATTAGA	

Table 2. Cartagena amplification conditions for PCR multiplex of tested loci.

	STR locus	Primers [ ] (µM)	Reactive	Final [ ]	PCR conditions	Temp ˚C	Time	Cycles
Multiplex 1	DYS391	0.8	Buffer	1X	Pre-denaturalization	95	10 min	
	DYS437	0.7	$MgCl_2$	1.75 mM	Denaturalization	94	1 min	
	DYS439	0.048	dNTP	200 mM	Annealing	55	1 min	30
	DYS448	0.016	Betaina	0.83 M	Extension	70	1 min	
	DYS458	0.9	TaqPol	1 U	Post-extension	60	45 min	
Multiplex 2	DYS19	1.2	Buffer	1X	Pre-denaturalization	95	10 min	
	DYS393	0.2	$MgCl_2$	1.75 mM	Denaturalization	94	1 min	
	DYS438	0.25	dNTP	100 mM	Annealing	55	1 min	30
	GATA-H4	0.4	Betaina	0.83 M	Extension	70	1 min	
			TaqPol	1 U	Post-extension	60	45 min	
Multiplex 3	DYS389I	0.25	Buffer	1X	Pre-denaturalization	95	10 min	
	DYS389II	0.25	$MgCl_2$	1.75 mM	Denaturalization	94	1 min	
	DYS390	0.2	dNTP	100 mM	Annealing	55	1 min	30
	DYS392	0.5	Betaina	0.83 M	Extension	70	1 min	
	DYS456	0.2	TaqPol	1 U	Post-extension	60	45 min	
	DYS635	0.5						

Table 3. Cartagena de Indias haplogroup identification from haplotype definition, with fitness score and probability.

Haplotype #	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y GATA-H4	Haplogroup	Fitness Score	Probability
hc-16	14	13	29	24	11	12	15	17	10	-	20	18	17	21	12	I2b1	16	0.682
hc-23	14	13	28	25	11	12	12	17	10	12	19	14	16	21	11	L	27	0.778
hc-24	13	14	30	25	11	14	13	16	13	12	19	16	17	23	12	R1b	31	0.981
hc-30	15	14	28	24	11	12	13	15	10	13	21	15	17	22	12	I2a1	34	0.864
hc-33	13	13	29	23	11	12	12	18	10	12	20	16	14	19	11	L	14	0.623
hc-40	14	13	29	24	11	12	13	17	10	13	20	16	19	21	11	E1b1b	25	0.503
hc-42	-	14	30	22	-	12	-	-	-	-	20	17	-	21	-	I2b1	54	0.95
hc-67	-	14	29	25	-	14	-	-	-	13	19	16	-	23	-	R1b	33	0.843
hc-89	15	15	31	24	11	13	14	15	10	11	20	16	15	22	11	I2b1	31	0.936
hc-95	14	13	29	29	11	13	13	15	12	12	19	16	16	23	12	R1b	33	1
hc-111	-	14	29	25	12	14	13	16	-	12	19	17	16	23	-	R1b	28	0.995
hc-113	15	15	32	23	11	12	13	15	11	12	21	16	16	21	10	E1b1a	32	0.771
hc-115	13	14	30	25	12	14	12	16	11	13	19	16	16	23	13	Q	20	0.687
hc-120	14	14	30	23	11	12	14	17	11	11	20	17	17	21	11	I2b1	23	0.916
hc-142	13	14	29	25	11	14	14	16	13	12	19	16	19	23	12	R1b	18	0.879
hc-149	14	14	29	23	11	14	13	16	13	11	19	16	18	23	12	R1b	31	1
hc-159	-	14	30	25	11	14	-	16	-	-	19	16	17	23	-	R1b	44	0.993
hc-161	13	16	31	24	11	12	12	15	9	11	20	17	15	22	11	J2a1 x J2a1-bh	19	0.906
hc-162	-	13	-	25	12	14	-	16	-	13	19	16	16	23	-	R1b	31	0.991
hc-165	-	14	31	25	10	12	-	15	-	12	20	16	16	23	-	E1b1b	42	0.388
hc-168	13	15	30	25	13	14	13	16	13	11	19	16	18	23	11	R1b	14	1
hc-169	14	13	28	25	11	12	12	17	-	12	-	14	16	21	11	J2b	17	0.843
hc-170	-	14	29	25	11	14	-	15	-	-	18	15	18	24	-	R1b	30	0.979
hc-172	-	14	30	22	11	12	-	15	-	11	21	16	-	21	-	J2a1 x J2a1-bh	38	0.353
hc-179	-	13	29	23	11	12	-	15	-	-	-	16	16	22	-	I2b1	47	0.462
hc-184	-	15	-	24	11	15	13	15	-	13	19	17	15	22	11	Q	35	0.983
hc-185	-	14	30	25	11	14	-	16	-	12	-	16	17	23	-	R1b	44	0.997
hc-186	-	-	-	23	11	12	13	15	-	13	21	16	18	22	-	I2a (xI2a1)	40	0.522
hc-191	14	13	29	23	11	13	13	15	12	13	19	17	17	23	13	R1b	58	1
hc-200	-	15	31	22	11	12	15	15	-	12	21	17	17	21	10	I2b1	24	0.841
hc-202	-	13	28	25	11	12	-	17	-	12	-	14	17	22	-	I2a (xI2a1)	23	0.688
hc-204	15	14	30	22	11	12	13	15	11	12	22	15	15	21	13	J2a1 x J2a1-bh	23	0.68
hc-219	-	13	-	25	11	14	-	15	-	12	18	19	17	23	-	R1b	36	0.991

Continued

hc-221	12	14	29	24	11	15	12	16	11	-	-	17	17	23	12	Q	16	0.534
hc-223	-	15	31	23	-	-	-	-	-	-	-	16	-	21	-	T	51	0.464
hc-230	-	-	-	-	11	-	-	16	-	-	20	-	15	-	-	I1	57	0.873
hc-231	14	14	-	22	13	-	13	15	11	11	21	16	16	21	12	E1b1a	19	0.845
hc-232	13	14	31	25	13	14	13	15	-	12	19	17	15	23	-	R1b	20	0.96
hc-233	13	14	29	25	11	14	13	16	13	12	19	16	17	24	12	R1b	23	0.974
hc-235	13	14	29	25	11	14	13	16	13	12	19	17	17	23	12	R1b	25	0.958
hc-238	15	14	30	26	11	12	13	15	11	11	20	18	15	23	13	R1a	30	1
hc-240	13	14	29	26	12	14	14	16	13	12	19	18	19	24	12	R1b	9	0.827
hc-251	-	14	28	25	12	14	-	16	-	12	-	17	17	23	-	R1b	17	0.987
hc-254	13	15	-	24	13	15	13	15	-	13	20	17	16	23	12	R1b	11	0.914
hc-255	13	-	-	-	11	-	13	16	9	11	22	-	16	-	11	J2b	15	0.357
hc-266	13	13	29	25	11	12	13	15	10	11	20	16	14	21	11	E1b1b	36	0.969
hc-268	16	14	30	22	11	12	14	15	11	13	21	17	16	21	11	E1b1a	27	0.573
hc-269	12	14	30	25	11	14	13	15	10	13	21	17	15	23	12	Q	20	0.945
hc-271	13	14	29	26	11	15	13	16	13	13	19	16	16	23	12	R1b	15	0.829
hc-274	13	14	29	26	12	14	13	16	13	12	19	16	17	23	12	R1b	17	0.99
hc-278	14	13	29	24	11	13	13	15	10	13	19	16	17	23	12	R1b	53	1
hc-280	-	13	30	22	11	12	-	15	-	12	-	16	18	21	-	J2a1b	44	0.796
hc-284	-	-	-	-	13	-	13	16	-	12	20	-	17	-	-	I2a (xI2a1)	24	0.791
hc-285	14	-	-	-	13	-	13	18	8	11	-	-	17	-	9	I2b (xI2b1)	8	0.993
hc-288	-	14	30	22	11	13	-	15	-	12	-	16	19	21	-	T	45	0.519
hc-291	12	15	30	24	13	14	13	15	13	13	18	17	17	23	11	R1b	14	1
hc-300	-	15	-	25	13	14	-	16	-	-	-	16	17	24	-	R1b	9	0.98
hc-308	14	14	29	24	11	15	13	15	9	12	19	15	17	21	11	T	51	1
hc-309	-	15	-	24	11	15	-	15	-	-	19	15	17	21	-	T	32	0.692
hc-310	12	14	31	25	11	13	13	15	10	11	21	18	17	23	12	R1b	14	0.484
hc-314	-	15	30	26	12	15	-	16	-	11	-	17	17	23	-	R1b	9	0.979
hc-315	14	14	29	24	11	15	13	15	9	12	19	15	17	21	11	T	51	1
hc-317	-	14	30	25	11	14	-	14	-	-	-	17	15	22	-	Q	48	0.646
hc-347	-	14	28	24	11	12	13	16	-	11	21	15	15	24	-	I2a (xI2a1)	24	0.899
hc-350	-	14	28	24	11	-	-	16	-	-	-	15	15	24	-	L	28	0.747
hc-356	17	13	28	23	10	11	13	15	10	11	21	14	15	24	12	I2a1	66	1
hc-358	-	14	28	24	11	12	-	15	-	11	21	15	15	24	-	I2a (xI2a1)	29	0.517
hc-360	15	13	32	21	10	11	13	14	11	11	20	15	16	21	12	E1b1a	66	1,00
hc-362	14	13	29	23	10	10	15	16	11	11	19	15	17	25	12	L	32	0.998

Continued

hc-364	15	14	30	24	11	13	13	14	12	12	19	15	17	23	12	R1b	57	1
hc-365	-	14	30	25	12	12	13	16	-	12	19	16	18	24	12	R1b	26	0.995
hc-373	-	14	30	25	-	14	-	-	-	-	-	16	-	23	-	R1b	44	0.794
hc-376	-	14	-	25	13	14	-	16	-	-	-	16	16	23	-	R1b	14	0.964
hc-377	13	14	30	25	-	-	12	-	13	13	-	16	-	23	12	R1b	20	0.613
hc-378	-	14	29	25	10	12	-	15	-	-	-	17	18	21	-	I2a (xI2a1)	29	0.411
hc-388	12	14	30	26	11	13	13	15	-	-	-	17	17	22	12	L	27	0.778
hc-390	-	15	33	24	11	16	-	15	-	-	-	16	15	22	-	R1b	31	0.981
hc-391	-	14	33	24	11	16	-	-	-	-	-	16	15	22	-	I2a1	34	0.864
hc-392	-	14	33	24	11	16	-	15	-	-	-	16	15	22	-	L	14	0.623
hc-393	-	14	33	24	11	16	-	15	-	-	-	16	15	22	-	E1b1b	25	0.503
hc-394	13	14	31	25	13	14	13	15	13	-	-	17	15	23	12	I2b1	54	0.95
hc-395	-	13	30	23	11	12	-	17	-	-	21	16	17	20	-	R1b	33	0.843
hc-396	13	16	32	23	11	12	13	17	10	12	19	15	17	24	12	I2b1	31	0.936
hc-398	14	15	31	23	11	12	13	15	10	11	20	16	17	22	11	R1b	33	1
hc-399	-	15	-	25	13	14	-	16	-	-	-	16	17	23	-	R1b	28	0.995
hc-402	12	-	-	-	11	-	13	15	-	12	19	-	15	-	11	E1b1a	32	0.771
hc-403	-	14	29	24	-	12	-	-	-	-	-	18	-	21	-	Q	20	0.687
hc-404	-	14	31	26	11	12	-	15	-	12	-	18	15	20	-	I2b1	23	0.916
hc-405	13	14	29	24	12	14	12	16	13	12	18	16	18	24	12	R1b	18	0.879
hc-410	-	15	30	26	10	12	-	15	-	-	-	15	18	21	-	R1b	31	1,00
hc-412	-	14	30	25	11	12	-	15	-	-	-	17	15	23	-	R1b	44	0.993
hc-413	-	14	31	22	11	12	-	15	-	-	-	16	17	22	-	J2a1 x J2a1-bh	19	0.906
hc-414	-	14	30	25	11	12	-	15	-	-	-	16	19	21	-	R1b	31	0.991
hc-415	-	14	29	24	11	12	-	15	-	13	-	16	21	21	-	E1b1b	42	0.388
hc-416	-	15	29	24	12	14	-	16	-	12	-	16	17	23	-	R1b	14	1,00
hc-418	-	14	29	22	11	12	-	15	-	-	-	18	14	21	-	J2b	17	0.843
hc-419	-	14	29	25	12	14	-	16	-	-	-	17	18	23	-	R1b	30	0.979
hc-420	-	14	30	23	11	12	-	15	-	12	-	16	16	21	-	J2a1 x J2a1-bh	38	0.353
hc-421	-	14	31	24	11	12	-	16	-	11	-	16	14	20	-	I2b1	47	0.462
hc-422	14	13	29	25	11	13	13	15	12	11	19	16	18	23	13	Q	35	0.983
hc-423	13	14	29	26	11	15	13	16	13	-	-	17	19	23	12	R1b	44	0.997
hc-424	-	15	31	24	11	14	-	15	-	12	-	18	16	21	-	I2a (xI2a1)	40	0.522
hc-425	-	13	29	25	11	12	-	15	-	-	20	16	18	21	-	R1b	58	1
hc-426	-	-	-	-	11	12	13	15	-	11	21	-	15	-	-	I2b1	24	0.841
hc-427	16	13	30	23	11	13	12	18	-	11	-	16	14	20	11	I2a (xI2a1)	23	0.688

Continued

hc-428	-	15	30	24	13	14	13	15	-	11	20	16	17	23	-	J2a1 x J2a1-bh	23	0.68
hc-430	14	14	30	24	12	14	13	14	9	11	19	15	16	21	11	Q	16	0.534
hc-431	-	14	29	26	14	14	-	16	-	-	-	16	19	23	-	T	51	0.464
hc-432	13	14	29	25	12	14	13	16	13	12	19	17	18	23	12	I1	57	0.873
hc-433	-	-	-	-	12	14	-	15	-	11	-	-	19	-	-	E1b1a	19	0.845
hc-434	12	15	30	25	10	12	13	15	10	10	19	17	18	22	12	R1b	20	0.96
hc-435	-	13	28	24	11	12	-	17	-	12	20	15	16	23	-	R1b	23	0.974
hc-436	13	14	29	26	12	14	13	16	13	14	19	18	18	24	11	R1b	25	0.958
hc-437	13	15	30	23	13	14	13	15	13	12	18	17	18	22	11	R1a	30	1
hc-438	-	14	29	25	12	12	13	16	-	12	19	17	17	24	-	R1b	9	0.827
hc-439	-	14	-	26	13	-	-	17	-	-	-	17	17	24	-	R1b	17	0.987
hc-440	14	14	30	24	10	13	13	15	12	12	19	16	17	23	12	R1b	11	0.914
hc-441	13	14	29	24	12	14	13	16	13	12	19	18	16	23	12	J2b	15	0.357
hc-444	-	15	30	26	12	14	-	16	-	-	-	19	16	23	-	E1b1b	36	0.969
hc-445	-	14	-	22	11	12	-	15	-	12	-	15	15	22	-	E1b1a	27	0.573
hc-446	15	13	31	21	10	11	13	14	11	12	21	14	15	22	10	Q	20	0.945
hc-447	13	14	29	24	11	11	14	17	11	-	-	16	17	25	12	R1b	15	0.829
hc-449	13	13	30	25	10	11	13	14	10	11	20	17	16	22	12	R1b	17	0.99
hc-450	-	14	32	22	11	12	-	15	-	12	-	16	17	21	-	R1b	53	1
hc-451	16	15	31	22	11	-	15	15	11	-	-	16	17	20	10	J2a1b	44	0.796
hc-453	-	15	31	25	11	14	13	16	-	12	19	17	17	23	-	I2a (xI2a1)	24	0.791
hc-454	-	14	28	24	11	-	-	16	-	-	-	14	15	23	-	I2b (xI2b1)	8	0.993
hc-455	14	13	28	23	11	12	13	17	10	-	-	15	14	22	11	T	45	0.519
hc-457	15	14	30	24	10	13	13	15	12	12	19	15	17	24	11	R1b	14	1
hc-458	14	14	32	21	11	12	13	15	11	12	20	16	16	21	12	R1b	9	0.98

In order to know the haplotype distribution, locus diversity, and mean number of pairwise differences, we used only the complete haplotypes (n = 37). Out of 37 haplotypes studied, we found 36 different haplotypes suggesting high haplotype diversity. In addition, locus diversity over loci showed the highest values (1.000 ± 0.0063), as well as the mean number of pairwise differences (10.084 ± 4.7048), whereas average gene diversity over loci obtained was 0.6722 + 0.3485.

3.2. Genetic Structure

In order to know the genetic structure, we determined the frequency of haplogroups as well as fitness score and Bayesian probability using Haplogroup predictor software (**Table 5**). Our results showed that Cartagena de Indias was an admixture population represented by ~80% European, ~10% Amerindian and ~10% African. The most frequent haplogroups were R1b (~40%), I2a (xI2a1) (11%) and Q (~10%), as well as E1b1a (~5%) and E1b1b (~4%). Additional haplogroups, evident in the low and moderate frequencies, were also found (G2a ~1%, I1 2%, I2a1~ 3%, I2b ~2%, I2b1 ~ 9%, J2a1-bh ~2%, J2b ~1%, L ~5%, R1a, 1% and T ~5%).

3.3. Comparison with Other Populations

We compared our data with previous results obtained in other Colombian populations. As can be seen in **Figure 2**, Cartagena's population maintains a genetic relationship with Antioquia, Magdalena and other populations

Table 4. Allelic frequencies, descriptive statistical parameters and diversity index regarding the 15 STR loci of Cartagena de Indias population.

Allele	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	YGAT-AH4
n	66	122	111	123	114	102	78	124	60	91	79	123	123	123	68
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	0.017	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	0.083	-	-	-	-	-	0.015
10	-	-	-	-	0.07	0.01	-	-	0.267	0.011	-	-	-	-	0.059
11	-	-	-	-	0.64	0.02	-	-	0.233	0.297	-	-	-	-	0.338
12	0.106	-	-	-	0.158	0.402	0.115	-	0.1	0.505	-	-	-	-	0.515
13	0.394	0.205	-	-	0.123	0.098	0.756	-	0.3	0.176	-	-	-	-	0.074
14	0.303	0.59	-	-	0.009	0.343	0.077	0.048	-	0.011	-	0.049	0.049	-	-
15	0.136	0.189	-	-	-	0.088	0.051	0.508	-	-	-	0.154	0.203	-	-
16	0.045	0.016	-	-	-	0.039	-	0.323	-	-	-	0.455	0.195	-	-
17	0.015	-	-	-	-	-	-	0.097	-	-	-	0.244	0.35	-	-
18	-	-	-	-	-	-	-	0.024	-	-	0.063	0.081	0.13	-	-
19	-	-	-	-	-	-	-	-	-	-	0.481	0.016	0.065	0.008	-
20	-	-	-	-	-	-	-	-	-	-	0.253	-	-	0.041	-
21	-	-	-	0.024	-	-	-	-	-	-	0.177	-	0.008	0.26	-
22	-	-	-	0.106	-	-	-	-	-	-	0.025	-	-	0.171	-
23	-	-	-	0.138	-	-	-	-	-	-	-	-	-	0.382	-
24	-	-	-	0.285	-	-	-	-	-	-	-	-	-	0.122	-
25	-	-	-	0.333	-	-	-	-	-	-	-	-	-	0.016	-
26	-	-	-	0.106	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	0.108	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	0.342	0.008	-	-	-	-	-	-	-	-	-	-	-
30	-	-	0.324	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	0.144	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	0.045	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	0.036	-	-	-	-	-	-	-	-	-	-	-	-
k	6	4	6	7	5	7	4	5	6	5	5	6	7	7	5
h	0.721	0.574	0.742	0.766	0.545	0.701	0.406	0.626	0.767	0.625	0.668	0.7	0.775	0.74	0.612

n: number of individuals studied; *k*: number of alleles; *h*: locus diversity.

Table 5. Cartagena de Indias haplogroup frequencies.

Haplogroup	Frequency
E1b1a	0.045
E1b1b	0.038
G2a	0.007
I1	0.015
I2a (xI2a1)	0.114
I2a1	0.03
I2b (xI2b1)	0.015
I2b1	0.091
J2a1b	0.015
J2a1 × J2a1-bh	0.022
J2b	0.007
L	0.045
Q	0.099
R1a	0.007
R1b	0.381
T	0.053

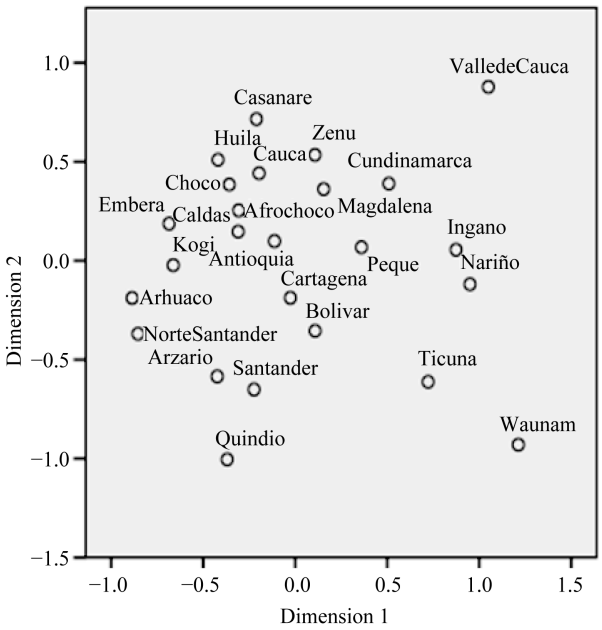

Figure 2. MDS plot of Colombian populations RST pairwise differences using 15 Y-STR loci.

from the Department of Bolivar. Moreover, Cartagena population is in the centre of the Colombian populations with different ancestries, underlining the complexity of this population.

3.4. Network Analysis

In order to establish the genetic relationship within each lineage, a median joining network was constructed

(**Figure 3**). The R1b haplogroup shows a star-like network, indicating that Cartagena's population is closely related to the Western European populations of Majorca and Valencia (Iberian Peninsula). In addition, a separate group of R1b Cartagena men related to Sicily's population, suggests a high genetic diversity even within this lineage (**Figure 3(a)**). Moreover, the I2a (xI2a1) lineage shows a star-like network suggesting that Cartagena's population could be related to a young population that may have suffered some demographic events (e.g. bottleneck, genetic drift, and founder effects). With respect to the Q haplogroup, we compared our data with Q-M242

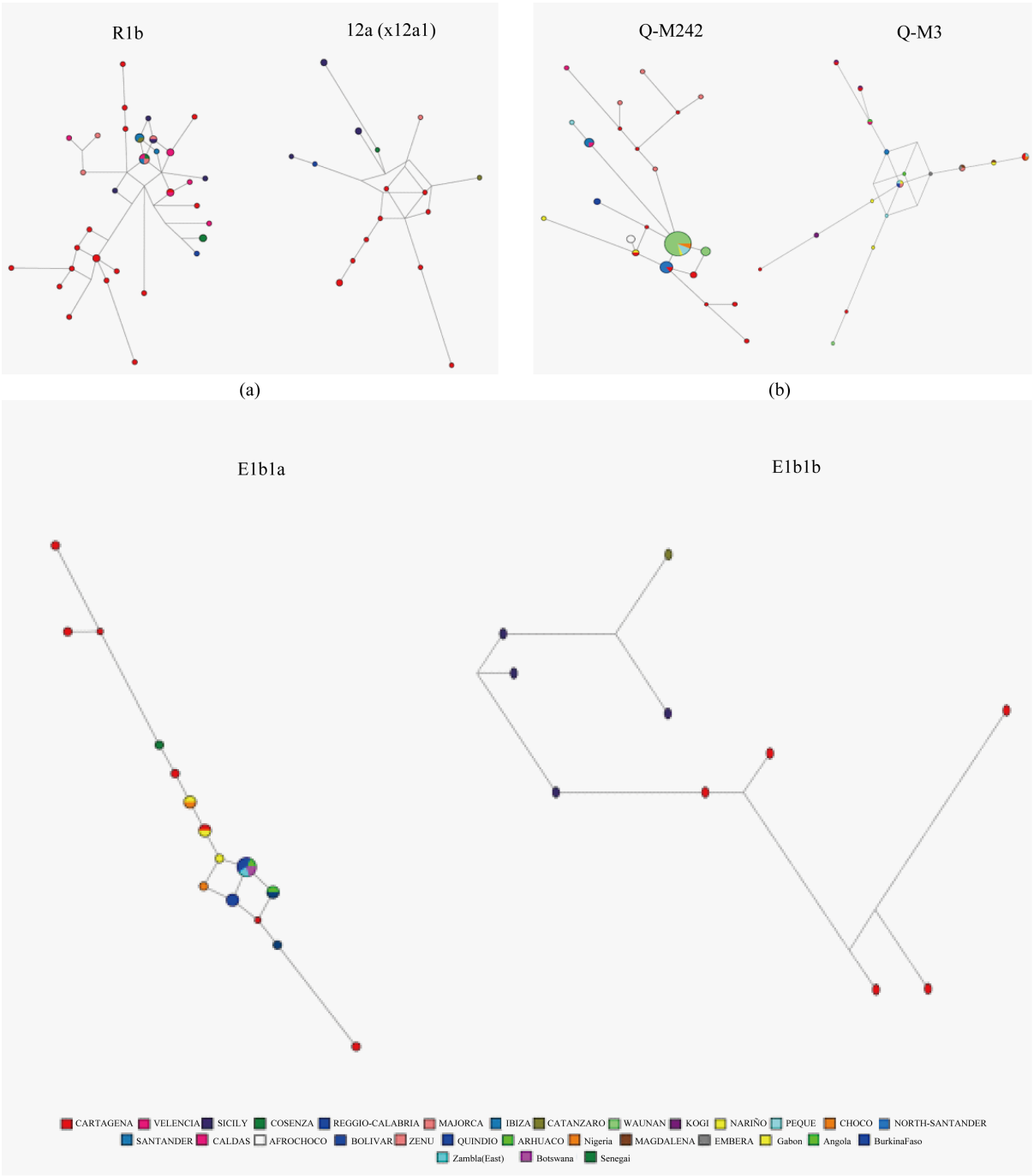

Figure 3. Median joining network of ancestral lineages in Cartagena de Indias population. (a) European lineages: R1b and I2a (xI2a1); (b) Amerindian lineages: Q-M242 and Q-M3; (c) African lineages: E1b1a and E1b1b.

and Q-M3 haplogroups described previously [3]. Cartagena's population showed a lineage closely related with Waunan (Q-M242) and Zenú ethnic groups. In addition, our haplogroups Q are related to Amerindian populations such as Kogi and Arhuacos (**Figure 3(b)**). Nevertheless, this diversity of pattern shows lineages poorly characterized by the lack of availability of markers that allow a higher resolution. Our results also point to the important African ancestry in Cartagena's population represented by E1b1a and E1b1b lineages (**Figure 3(c)**). Interestingly, Cartagena's population also has a patrilineal relationship with Senegal and Gabon.

4. Discussion

Population stratification is one of the most important confounding factors in population-based genetic association studies, provoking 40% of spurious associations [20]. These false associations are more frequent in Latino populations, heterogeneous populations in which a dissimilar ancestry proportion give rise to each subpopulation not being equally represented [21]-[24]. Consequently, in recent years much research has focused on detecting the population stratification before beginning genetic association studies of complex diseases in order to avoid spurious associations [5] [25] [26].

The contemporary Cartagena de Indias population emerged from recent miscegenation (500 years ago) as a cosmopolitan city where Spanish conquerors mixed with Native American people derived principally from Karib, Malibu, Arawak and Chibcha language families [27]. As stated above, this admixture was asymmetrical and it is in agreement with our findings which show that the Cartagena sample studied is comprised of ~80% European, ~10% Amerindian and ~10% African ancestries. With respect to the European ancestry, it was principally represented by the haplogroup R1b (hg-R1b), which was present in ~50% of the total European haplogroups found in Cartagena's population. The hg-R1b is the result of the admixture with the Spanish conquerors during the colonial period, because Cartagena was one of the most important Spanish settlements in America [28] [29]. This haplogroup is actually present in more than 60% of the Spaniard population [30], as well as ~ 80% of Basque Country population [17] [31]. However, hg-R1b could be also related to Mediterranean populations [17], since it showed a haplotype relationship with Cartagena's population (**Figure 3(a)**). In addition, the Italian population could also participate in introducing the other important European lineage (haplogroup I2a (xI2a1)). This haplogroup is one of the most frequent in the island of Sardinia as well as the Mediterranean region [15], and could be related to the Italian migrations 200 years ago [28]. These Italian migrations came from Sicily and Cosenza principally, and settled down on the northern coast of Colombia (Barranquilla, Cartagena and Santa Marta) [32]. With respect to the other European lineages, these could be related to pirates and corsairs from England, France, Portugal and the Netherlands, who continuously invaded Cartagena de Indias because this city was the principal port for gold and silver during the colonial period [29] [33].

Apart from the European ancestry, Cartagena showed an important contribution from African lineages, which were represented by the haplogroups E1b1a and E1b1b. As mentioned before, thousands of African slaves especially those from Western and Central Africa were introduced in the 16th century [2], which disembarked on the Pacific and Atlantic Coasts [34]. The African lineages increased noticeably the diversity of Cartagena's population because they represented different clans from Senegambia, Ivory Coast, Central Africa, Congo, Angola and Mozambique among others; many of them were found in Cartagena's population [3] [35] (**Figure 3**).

Both ancestries (European and African) were admixed with native Amerindian populations, which actually maintained an ancestral relationship with Cartagena's population. On the other hand, our results suggested that the Amerindian diversity of Cartagena was related with Waunan, Kogui, Chocó, Pequé and Zenú groups, all of which were related to the Q-M242/Q-M3 lineages, which represented the majority of Amerindian Y chromosomes [36]. Nevertheless, the Amerindian diversity of Cartagena's population could show even more heterogeneity, which could be related to the diversity inside the Q haplogroup [3].

The great genetic diversity of Cartagena de Indias' population, represented by mestizo, Afro-Colombian, and Amerindian lineages, supports the importance of ancestral studies in admixture populations. Our results suggest an important substructure degree within Cartagena's population. This dissimilar ancestral proportion indicates the necessity to increase the resolution as well as the use of different genetic markers in order to elucidate the complex population history of Cartagena.

Although different research groups have also studied Colombian and Cartagena samples populations, the regional differences, the demographic events, and the complex patterns of diversity suggest examining different samples of the same population in order to represent the whole genetic complexity [5] [37]-[39]. Nevertheless,

these ancestral patterns should not be applied to the entire Colombian population, because the demographic events and consequently the diversity patterns are specific of each population.

In addition, our results emphasize the contribution of population genetics in population-based genetic association studies, where the ethnic self-identification is not appropriate to correct the population stratification. Our data could contribute to avoid or diminish statistical errors type 1 and 2, which is a fundamental strategy in the search of disease biomarkers.

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