

## **GENETIC POLYMORPHISMS AND RACIAL GROUPS IN THE POPULATION OF PINAR DEL RIO PROVINCE (CUBA)**

*Victor P. Díaz Narvaez<sup>1</sup>  
Pedro Hidalgo Calcines<sup>1</sup>  
Tomás Castellanos Pérez<sup>1</sup>  
Olga González González<sup>1</sup>  
Aracelis Calzadilla Nuñez<sup>2</sup>  
Carlos Valenzuela Yuraidimi<sup>3</sup>  
Margarita Rodríguez Pérez<sup>1</sup>*

**PALABRAS CLAVE:** Marcadores genéticos, Polimorfismo, Grupos raciales, Pinar del Río, Cuba

**RESUMEN:** Se estudió el polimorfismo genético de 13 marcadores genéticos en tres grupos raciales (blancos, mulatos y negros) en la provincia de Pinar del Río (Cuba). Se encontraron diferencias fenotípicas en 8 de los 13 marcadores estudiados. Las diferencias de las frecuencias alélicas encontradas en los marcadores fueron altamente significativas entre los grupos raciales examinados. Los loci estudiados permitieron mostrar claras diferencias de un grupo racial respecto de los otros. Los hallazgos obtenidos permiten confirmar la hipótesis de que la población estudiada no es homogénea. *Rev. Arg. Antrop. Biol.* 4(1): 9-20, 2002.

**KEY WORDS:** Genetic markers, Polymorphism, Racial groups, Pinar del Río, Cuba

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<sup>1</sup> Laboratorio de Genética Humana. Departamento de Investigaciones. Instituto Superior de Ciencias Médicas de Santa Clara. Villa Clara, Cuba.  
e-mail: vpdiaz@entelchile.net

<sup>2</sup> Departamento de Investigaciones. Facultad de Ciencias Médicas de Pinar del Río. Pinar del Río, Cuba.

<sup>3</sup> Programa de Genética Humana. ICBM. Facultad de Medicina. Universidad de Chile. Santiago, Chile.

**ABSTRACT:** The polymorphism of 13 genetic markers in three racial groups (whites, mulattos and blacks) of the population of Pinar del Río province (Cuba) was investigated. Differences among phenotypic rates in 8 of the 13 markers studied were found. The allelic frequencies in the markers that showed differences were highly significant among the racial groups. The loci investigated were able to clearly differentiate one racial group from the others. This finding confirms the presumption that the population of this region is not homogeneous. *Rev. Arg. Antrop. Biol.* 4(1): 9-20, 2002.

## INTRODUCTION

The Pinar del Río province is located in the western tip of the Island of Cuba. Its population is near 697.986 inhabitants (Censo de Población y Viviendas, 1981) and occupies an area of 10.924,56 Km<sup>2</sup>.

The population of this province, as well as that from Cuba, is the product of migrations of different racial groups: whites of Spanish origin, negroes from Africa and Chinese from Asia. In 1492 this province was the least populated region in Cuba.

This part of Cuba was originally inhabited by aborigines of the Guanahatabey cultural group (Carreras, 1985). The onset of Spanish colonization originated a racial mixture between whites and aborigines. Towards the end of the XVIth century, few traces of the Guanahatabey cultural group remained (Pérez de la Riva, 1972; Alonso, 1990). The Spaniards that colonized this region, during the first decade of the XVth century, were primarily from Canary Islands and Castilla (Le Riverend, 1974; Sohegui, 1980).

African Negroes were brought to Cuba as slaves since the Spaniards settlement and during colonization (XVth century) (Moreno Fragnals, 1978). The number of slaves brought to Cuba during colonization is thought to be over one million (Pérez de la Riva, 1970). The exact origin of this population is unknown, but it is believed that most of them were taken from places along the west coast of Africa (from Senegal to Angola) (Moreno, 1976; Moreno Fragnals, 1977; Rivero de la Calle, 1981). The racial admixture between whites and blacks created the "mulatto" group. This group is a typical element in the Cuban population (Hidalgo, 1986). The Chinese people were brought to Cuba in the middle of the XIXth century from Kwangtung and Fukien provinces. Despite the great number of Chinese coolies who arrived in Cuba (124.000), only 49 were females, according to the 1899 Census (Pérez de la Riva, 1967). Consequently, the Asian immigration left only a few traces on the structure of the Cuban population. This group represents a small fraction of the studied population and its possible contribution was not taken into account.

Studies of genetic markers in the Cuban population are scarce. They are limited mainly to Mas-Martín et al. (1964) (ABO and RH (D)) in Habana City (capital of Cuba), González et al. (1976), García et al. (1982) (GLO1), Barrios and Granda (1983) and Hidalgo (1986) in the central region of Cuba. In Pinar del Río province only our studies on the ABO (without A1 and A2 phenotypes) (Díaz, 1985) and HP systems (Díaz et al., 1995) have been performed.

The objective of this work is to investigate the polymorphism distribution of 13 genetic markers in the population of Pinar del Río province and to determine whether there exist phenotypic and allelic differences in each genetic marker studied among the three typical racial groups of this population: whites, mulattos and blacks.

## **MATERIAL AND METHODS**

The sample was randomized and collected from unrelated volunteer blood donors, from Pinar del Río province Blood Banks. Among 95% donors were males and aged 20 to 40. All subjects were born in Pinar del Río, so were their parents and grandparents. The conditions of the study were communicated to all participants and each of them signed a written consent form authorizing their blood analysis.

To ensure the sample was representative of the province, the subjects were selected from each political-administrative region (municipalities) of the province.

The classification of subjects into racial groups (White, Mulatto and Black) was made according to two criteria: direct observations of morphologic characters (Hidalgo, 1986) and personal interviews where specific questions on the ancestry were asked to each of the participants by a single interviewer. We joined Mulattos and Blacks in a new group referred to as Negroids.

Blood samples were obtained by venous puncture with anticoagulant. Plasma and cells were separated by centrifugation. Then plasma was frozen at -30°C so as to study the CHE2, PI, GC and TF systems. The red blood cells were subjected to two different processes: 1) resuspension of erythrocytes, which was made to determine phenotypes of ABO (without A1 and A2), MN and P systems; 2) crioconservation, as described by Issitt (1985). This was undertaken for a later study of the HB, GLO1, PGM1 and G6PD systems, A1 and A2 phenotypes of ABO system and phenotypes of KELL and RH (haplotypes) systems.

The samples were transferred from the Pinar del Río Blood Bank to the Human Genetic Laboratory of The Higher Medical Science Institute of Santa Clara, Villa Clara (Cuba), at -30°C.

The phenotype determination of ABO (without A1 and A2), MN and P systems was made by the method described by Levine (1954). The KELL, RH (haplotypes), and A1 and A2 phenotypes were studied by the microenzymatic technique described by The American Association of Blood Banks (1985). The phenotype determination of Hemoglobin (HB), Glucose-6-Phosphate Dehydrogenase (G6PD) and Pseudocholinesterase (CHE2) systems was typed according to the methods described by Ciscar (1972), Beutler (1966) and Heredero et al. (1974), respectively; and the phenotypes of Glyoxalase I (GLO1), according to Taggart et al. (1978), and Palmour et al. (1980). The phenotypes of Alpha-1-antitripsin (PI) system were determined by the method described by Kueppers (1976). The phenotypes for the Group Specific Component (GC) and Transferrin (TF) were determined according to Dannewitz (1985), and the phenotypes of Phosphoglucomutase I (PGM1) system were obtained by the method published by Dykes and Polensky (1981).

The homogeneity of phenotype frequencies among racial groups was tested by the chi-square test of homogeneity ( $X^2$ ). The significance level was chosen at  $p < 0,01$  (negroids were excluded from this comparison).

The allelic frequency estimates were obtained by the maximum likelihood method. In systems with one completely dominant allele we assumed that the Hardy-Weinberg equilibrium was reached. In the estimation of haplotype frequencies of the RH system five sera were employed: anti-C, anti-c, anti-D, anti-E and anti-e, considering the simultaneous presence of Cde and CDE.

The Hardy-Weinberg equilibrium condition was calculated according to standard procedures described by Smith (1970). A  $p < 0,01$  level of significance with one degree of freedom was used. For those loci with more than two alleles, the expected genotypic proportions were obtained by means of an obvious extension of the Hardy-Weinberg law. In those cases, the degrees of freedom were calculated as the difference between the number of alleles and the number of possible phenotypes (Workman et al., 1963).

## RESULTS

The results of phenotypic and allelic frequencies of all genetic systems are presented in Tables 1 to 4 for whites, mulattos, blacks and negroids in general, respectively.

The  $X^2$  test for homogeneity of phenotypes among racial groups was highly significant ( $p < 0,01$ ) in the ABO, P, RH (haplotypes), TF, GC, G6PD, HB and GLO1 (Table 5).

The  $X^2$  (H-W) test was not significant for all the racial groups and (for all the) genetics markers (Tables 1 to 4).

## **DISCUSSION**

If all genes with a frequency higher than 0,01 are considered polymorphic (Harris, 1980), it can be stated, according to our study, that hemoglobin is not polymorphic in the white and black groups. Moreover, certain systems such as CHE2 and TF show polymorphic borderline frequencies.

The differences observed in the allelic frequencies between the different racial groups are important. Thus, the Pinar del Río population is not homogeneous. These findings are in agreement with previous results obtained by González et al. (1976); Hidalgo (1986); García et al. (1982); Barrios and Granda (1983) in other Cuban populations.

Upon analyzing the heterogeneity between the different racial groups it was observed among the 13 systems that most of them showed significant differences. If data about HP (haptoglobin) system obtained by Díaz et al. (1995) in the same population are included, we can see that 9 out of 14 genetic systems in that population showed highly significant differences. This racial heterogeneity has been maintained by more than 17 generations since the Spaniard's arrival to Cuban Islands. Possibly, assortative mating maintains these groups apart.

The non-significant deviation from the Hardy-Weinberg equilibrium obtained by the goodness-of-fit test between the observed and the expected phenotypic frequencies suggests that the conditions of random mating, homogeneity of subsamples, and absence of selection are roughly fulfilled in each group.

These findings confirm our hypothesis about the existence of differences between racial groups in Pinar del Río. Taken together, these loci discriminate well among the racial groups examined here (64,4% of these polymorphisms studied in our population showed significant differences).

## **ACKNOWLEDGEMENTS**

The authors are grateful to Dr. Luciano Terrenato, Univ. Tor Vergata Roma for the helpful criticisms of this paper.

Table 1

Phenotypic and allelic frequencies in each genetic system studied in Whites

ABO	KELL	GLOI	PI	TF
A1	50	1-1	MM	1-1
A2	20	2-1	MS	2-2
B	6	2-2	SS	3-3
A1B	4	n	PI*M	1-3
A2B	0	n	PI*S	2-3
O	72	GLO1*1	$\chi^2 = 1,37; 1 \text{ d.f.}; p > 0,01$	1-2
n	152	GLO1*2	n	n
ABO*A1	0,1962±0,02412	$\chi^2 = 0,36; 1 \text{ d.f.}; p > 0,01$	n	n
ABO*A2	0,0859±0,01842			TF*C1
ABO*B	0,0333±0,01038			TF*C2
ABO*O	0,6844±0,02902			TF*C3
$\chi^2 = 3,19; 2 \text{ d.f.}; p > 0,01$				$\chi^2 = 1,08; 3 \text{ d.f.}; p > 0,01$
MM	52	G6PD	PGMI	GC
MM 52		Normals	1A	IF
MN 97		Deficients	1B	IS
NN 32		G6PD**	1A1B	2-2
n 181		G6PD*-	2A	2-1F
MN*M	0,5552±2,6118E-2	n	2B	2-1S
MN*N	0,4448±2,6118E-2	n	2A2B	IF-1S
$\chi^2 = 1,30; 1 \text{ d.f.}; p > 0,01$		HB	1A2A	n
		AA	1A2B	n
		AS	1B2A	GC*IF
		AC	1B2B	GC*1S
		n	n	GC*2
		HB*A	n	$\chi^2 = 11,42; 3 \text{ d.f.}; p > 0,01$
		HB*S	PGM*1A	
		$\chi^2 = 0,007; 1 \text{ d.f.}; p > 0,01$	PGM*1B	
P		CHE2	PGM1*2A	
P1 136		C5+	PGM*2B	
P2 16		C5-	$\chi^2 = 9,10; 6 \text{ d.f.}; p > 0,01$	
n 152		n		
P*1 0,6755±0,0383		CHE2*-		
P*2 0,3245±0,0383		CHE2**		
		$\chi^2 = 1,81; 3 \text{ d.f.}; p > 0,01$		

 $\chi^2$ : Chi-Square of Hardy-Weinberg equilibrium



**Table 3**  
Phenotypic and allelic frequencies in each genetic system studied in Blacks

ABO	KELL	GLO1	PI	TF
A1	KK 2	1-1 7	MM 98	1-1 99
A2	Kk 25	2-1 48	MS 7	2-2 0
B	kk 186	2-2 51	SS 0	3-3 0
A1B	n 213	n 106	PI* <b>M</b> 0,9667±0,0123	1-3 0
A2B	KEL*K 0,0681±0,0122	GLO1*1 0,2924±0,0312	PI*S 0,0333±0,0123	2-3 0
O	KEL*k 0,9319±0,0122	GLO1*2 0,7075±0,0312	χ <sup>2</sup> = 0,10; 1 d.f.; p>0,01	1-2 6
n	χ <sup>2</sup> = 1,19; 1 d.f.; p>0,01	χ <sup>2</sup> = 0,84; 1 d.f.; p>0,01	n 105	n 153
ABO*A1 0,0936±0,02042			TF*C1 0,9715±0,0114	
ABO*A2 0,0596±0,01728			TF*C2 0,0285±0,0114	
ABO*B 0,1045±0,02151			χ <sup>2</sup> = 7,73E-2; 1 d.f.; p>0,01	
ABO*O 0,7420±0,03121				
χ <sup>2</sup> = 4,52; 2 d.f.; p>0,01				
MN	CcDce 15	G6PD	PGMI	GC
MM 40	Ccddee 1	Normals 88	IA 42	1F 49
MN 67	ccDEE 1	Deficients 15	IB 2	IS 5
NN 34	ccDDe 28	G6PD*+ 0,8544±0,0246	1A1B 23	2-2 2
n 141	ccDde 49	G6PD*- 0,1456±0,0246	2A 4	2-1F 6
MN*M 0,5213±0,0297	Ccddee 6	n 103	IB2A 4	2-1S 3
MN*N 0,4787±0,0297	ccddEe 0	n 211	1B2B 1	1F-1S 40
χ <sup>2</sup> = 0,32; 1 d.f.; p>0,01	n 107	HB*A 0,9620±0,29E-3	n 105	n 105
P	Haplotypes	HB*S 0,0355±9,01E-3	PGM*1A 0,6429±0,0330	GC*1F 0,6857±3,2034E-2
P1 78	CDe 0,0911±0,02631	HB*C 0,0023±2,36E-3	PGM*1B 0,1524±0,0248	GC*1S 0,2523±2,9974E-2
P2 29	Cde 0,01888±0,01803	χ <sup>2</sup> = 0,32; 1 d.f.; p>0,01	PGM1*2A 0,1619±0,0254	GC*2 0,0619±1,6629E-2
n 107	CDE 0,00816±0,00009	CHE2	PGM*2B 0,0428±0,0139	χ <sup>2</sup> = 8,12; 3 d.f.; p>0,01
P*1 0,4795±0,0412	cDE 0,15071±0,02533	C5+ 3	χ <sup>2</sup> = 1,97; 6 d.f.; p>0,01	
P*2 0,5205±0,0412	cDe 0,00000±0,00004	C5- 103		
	cDe 0,48289±0,05009	n 105		
	cde 0,24023±0,04666	CHE2*- 0,9856±0,0082		
	χ <sup>2</sup> = 7,96; 3 d.f.; p>0,01	CHE2*+ 0,0144±0,0082		

χ<sup>2</sup>: Chi-Square of Hardy-Weinberg equilibrium

**Table 4**

Phenotypic and allelic frequencies in each genetic system studied in Negroids in general

ABO	KELL	GLO1	PI	TF
A1	49	1-1	MM	1-1
A2	26	2-1	MS	2-2
B	28	2-2	SS	3-3
A1B	2	n	PI*M	1-3
A2B	0	GLO1*1	PI*S	2-3
O	112	GLO1*2	$\chi^2 = 1,27; 1 \text{ d.f.}; p > 0,01$	1-2
n	217	$\chi^2 = 4,39; 1 \text{ d.f.}; p > 0,01$	n	216
ABO*A1	0,1257±0,01647			TF*CI
ABO*A2	0,0721±0,01356			0,9421±0,0112
ABO*B	0,0721±0,01265			TF*C2
ABO*O	0,7299±0,02247			0,0579±0,0112
$\chi^2 = 4,23; 2 \text{ d.f.}; p > 0,01$				$\chi^2 = 0,12; 1 \text{ d.f.}; p > 0,01$
MN		<b>G6PD</b>	<b>PGMI</b>	<b>GC</b>
MM	82	Normals	1A	1F
MN	148	Deficients	1B	1S
NN	64	G6PD*+	1A1B	2-2
n	294	G6PD*-	2A	2-1F
MN*M	0,5306±2,0580E-2	n	2B	2-1S
MN*N	0,4693±2,0580E-2	n	2A2B	1F-1S
$\chi^2 = 0,03; 1 \text{ d.f.}; p > 0,01$		HB	1A2A	n
		AA	1A2B	216
		AS	1B2A	GC*1F
		AC	1B2B	0,6158±0,0234
		n	n	GC*1S
		HB*A	PGM*1A	0,3218±0,0224
		HB*S	PGM*1B	0,1475±0,0170
		HB*C	PGM*2A	0,0625±0,0116
		$\chi^2 = 0,28; 1 \text{ d.f.}; p > 0,01$	PGM*2B	$\chi^2 = 6,67; 3 \text{ d.f.}; p > 0,01$
		<b>CHE2</b>	$\chi^2 = 1,57; 6 \text{ d.f.}; p > 0,01$	
P	155	C5+		
P1	62	C5-		
n	217	n		
P*1	0,4655±0,0286	CHE2*-		
P*2	0,5345±0,0286	CHE2*+		
		$\chi^2 = 13,94; 3 \text{ d.f.}; p > 0,01$		

$\chi^2$ : Chi-Square of Hardy-Weinberg equilibrium

**Table 5**

Chi-Square of homogeneity among whites, mulattos and blacks in each genetic system studied

System	$\chi^2$	d.f.	p
ABO	27,15	8	<0,001
MN	2,36	4	>0,05
KELL	4,52	4	>0,05
P	17,74	2	<0,0001
RH	101,61	18	<0,0001
CHE2	4,93	2	>0,05
PI	11,48	4	>0,01
TF	31,85	8	<0,001
HB	15,46	2	<0,0005
G6PD	13,81	2	<0,005
GC	181,21	10	<0,0001
GLO1	13,74	4	<0,01
PGM1	10,90	18	>0,05
HP	15,15	4	<0,01

(Díaz et al., 1995)

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