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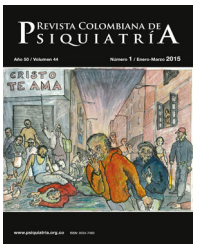

Original Article

Polymorphisms of apolipoprotein E in the Afro-descendant population of Buenaventura, Colombia

*Vivian Andrea Perdomo, Diana Carolina Ortega, Guillermo Barreto**

Grupo de Genética Molecular Humana, Departamento de Biología, Universidad del Valle, Cali, Colombia

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ABSTRACT

Objetives: To estimate the frequency distribution, both allelic and genotypic, of the *APOE* gene in the Afro-descendant population of Buenaventura, Colombia.
Methods: Three hundred and forty-eight Afro-descendant individuals were analysed and the *APOE* locus was genotyped by PCR-RFLP. The allelic and genotypic frequencies were established by direct counting and the Hardy-Weinberg equilibrium was evaluated through χ^2 test. The frequencies obtained in this study were compared with frequencies reported for other Colombian populations through the Fisher's exact test.
Results: The following allelic frequencies were observed: E3, 70.8%; E4, 21.4%, and E2, 7.8%. The genotypic frequencies were: E3/E3, 51.1%; E3/E4, 27.3%; E2/E3, 12.1%; E4/E4, 6%; E2/E4, 3.5%, and E2/E2, 0%. The entire examined population was found in Hardy-Weinberg equilibrium ($P = .074$), and significant differences were found in the allele E4 when comparing this population with the Amerindian and mestizo populations of Bogotá, Quindío, Centro-Oriente, Valle del Cauca, Barranquilla and Medellín ($P \leq 0.0345$).
Conclusions: The allelic frequencies observed in this study were significantly different from the frequencies reported in other Colombian populations. The high representativeness of the E4 and E2 alleles validates the hypothesis that there are micro-evolutionary processes that have been acting on their frequencies and could be associated with susceptibility to neuropsychiatric diseases such as Alzheimer's disease, metabolic alterations of fats and/or coronary artery disease.

* *Corresponding author.*
E-mail address: guillermo.barreto@correounivalle.edu.co (G. Barreto).

Polimorfismos del gen de la apopoliproteína E en la población afrodescendiente de Buenaventura, Colombia

Palabras clave:
Apolipoproteína E
Apo E4
Enfermedad de Alzheimer
Población afrodescendiente colombiana

RESUMEN

Objetivos: Estimar la distribución de frecuencias tanto alélicas como genotípicas del gen *APOE* en la población afrodescendiente de Buenaventura, Colombia.

Métodos: Mediante la técnica de PCR-RFLP's se analizaron 348 individuos no relacionados de esta ciudad. Se realizó el cálculo de frecuencias alélicas y genotípicas y se evaluó el equilibrio de Hardy-Weinberg mediante la prueba de la χ^2. Se compararon las frecuencias alélicas obtenidas en el presente estudio con otras poblaciones de Colombia mediante el test exacto de Fisher.

Resultados: Se reportaron las siguientes frecuencias alélicas: E2, 7,8%; E3, 70,8%, y E4, 21,4%. Las frecuencias genotípicas fueron: E3/E3, 51,1%; E3/E4, 27,3%; E4/E4, 6%; E2/E3, 12,1%; E2/E4, 3,5%, y E2/E2, 0%. La población total se encontró en equilibrio de Hardy-Weinberg ($p = 0,074$), y se hallaron diferencias significativas en el alelo E4 al comparar esta población con las amerindias y mestizas de Bogotá, Quindío, Centro-Oriente, Valle del Cauca, Barranquilla y Medellín ($p \leq 0,0345$).

Conlusiones: Las frecuencias alélicas observadas fueron significativamente diferentes de las frecuencias reportadas en otras poblaciones de Colombia. La alta representatividad de los alelos E4 y E2 validan la hipótesis de que hay procesos microevolutivos que han venido actuando en sus frecuencias y pueden estar asociadas con susceptibilidad a enfermedades neuropsiquiátricas como la enfermedad de Alzheimer, alteraciones metabólicas de las grasas y/o enfermedad coronaria.

Introduction

The gen of apolipoprotein E, as the main component of apoproteins in the chylomicron, codes a protein of 299 amino acids and has been used extensively as a genetic marker of risk in the development of Alzheimer's dementia type, and alterations in the way organism metabolizes fat.[1,2] Its fundamental role in the transport of lipoproteins and in the homeostatic maintenance of the lipids in organs as liver and brain[3] makes that his genotype be very important as a pathologic indicator.

The most common *APOE* alleles are E2, E3, and E4, which are differentiated by cysteine/arginine interchanges at codons 112 (TGC) and 158 (CGC) of its peptide.[4] There are numerous studies focused in the analysis of these gene variants and showing as each one affects the right function and expression of the gen and the physiologic impact that they are able to stimulate.[5-7]

In Caucasian Europeans, the allele E2 is associated with diabetes and hypercholesterolemia[1], but with a lesser risk of dementia,[8] contrary on data registered for the allele E4 which has been identified as a strong risk factor in several diseases including dyslipemia, atherosclerosis, coronary artery disease, and dementia type Alzheimer.[9] The isoform E3 with a frequency of 70-80% is associated to a normal function of the gene *APOE* in these populations.[10]

It is worldwide considered that the distribution of the allelic frequencies *APOE* follows the same tendency E3 >E4 >E2. However, different studies reassure the idea that the distribution of these polymorphisms differs from one population to another, being distinctive of each ethnic group, region and country.[11] The afrodescendant population characterizes for a considerable rise in the frequency of the allele E4 (while for the rest of the general population there are reports of frequencies less than 15%, the black people community presents a prevalence up to 25%),[12] in other way, the South American native's area is characterized by the absence of the allele E2.[13] The high frequency (>70%) of the allele E3 in the different populations studied might be influenced by biochemical factors, eating habits and the ethnic origin.[14]

Colombian studies with mestizo population from Bogotá, Antioquia and Barranquilla have shown that there are no significant differences in *APOE* allele frequencies for this population, possibly due to the genic flux between the Colombian center and north-east,[15] and possibly related too with Indo-European ancestry of this population.[16] When native population were considered, the APOE frequencies were different to the presented in mestizo population.[17,18] These studies clearly indicate that the *APOE* heterogeneity is not exclusive of Indo-European population, but it's also present in South American indigenous, whose absence of allele E2 is remarkable. Additionally, factors as population ancestry, immigration and admixture can explain this *APOE* heterogeneity in the different analyzed populations.

The afrodescendant population show patterns of ancestry differentiated from the other ethnic groups, that's why it is of interest for *APOE* heterogeneity study. There are not known studies in Colombia where the afrodescendant population are involved in an exclusive manner. The municipality of Buenaventura was founded in 1540, it is located in Department

Table 1 – Allelic and genotypic frequencies for the APOE locus observed in the Buenaventura's Afrodescendant population (n = 348).

Genotype							
E2/E2	E3/E3	E4/E4	E2/E3	E2/E4	E3/E4	Total	Equilibrium H-W
< .0001 Allele 2 .0776	.5115	.0603 Allele 3 .7083	.1207	.0345 Allele 4 .2141	.2730	1 Total 1	P = .0744

of Valle del Cauca, south western Colombia, it has a population of 415 640 inhabitants, with a component of 88.5% of afrodescendant,[19] which makes this population a reference point in the evaluation of *APOE* heterogeneity.

The high frequency of alleles E2 and E4 in individuals with afrodescendant origin which under these allelic variants have demonstrated susceptibility to metabolic alterations of fat, coronary artery disease and neuropsychiatric diseases, remarks the importance and necessity of establishing the frequency of these alleles. In this study, we aimed establish the *APOE* genotype and allele frequency, in a population mostly afrodescendant, as it is in the population of Buenaventura, located in south western Colombia. Our results were compared with allele frequencies for this gene reported for the other (amerindian, afrodescendent and mestizo) Colombian populations.

Methods

Molecular diagnosis and sampling

Study population were 348 Afrodescendant individuals, 72 males and 276 females, from 12 localities of the urban zone of Buenaventura, Colombia. The study excluded foreigners, visitors or residents who were not born in the city. In the same way, the participants weren't related among them in the same generation and there were only allowed 2 members of at least 2 different generations per family. From this sample, 70 individuals were under adult age (≤18 years) and 278 individuals were adults.

This investigation was approved by the Human Ethics Committee of the Universidad del Valle. Afterwards of the consent signature, a 4 mL sample of peripheral blood was taken from each participant. The DNA extraction was made by a modified "salting-out" method.[20] Genotyping of the *APOE* polymorphisms was performed by the digestion with restriction enzyme HhaI of 225 bp DNA fragment previously obtained by PCR. The digested fragments were separated in polyacrylamide gels at 8% dyed with silver nitrate. The RFLP's obtained were analyzed as published elsewhere.[21]

Statistical analysis

The frequencies determination of *APOE* was performed by direct counting. The analysis of Hardy-Weiberg equilibrium was done through the goodness of fit test χ^2 ($P < .05$) through the statistic package Arlequin 3.1.[22]

The allele frequencies comparison of *APOE* reported for Buenaventura (in the present study) with those observed in other Colombian populations, was carried out through the exact test of Fisher ($P < .05$) using the software R,[23] and also using other works.[11,15,17,24-29] In the valuation of these works were considered parameters as: *a)* ages under 65 years old, and *b)* avoiding studies of control cases with pathologic positive association.

Results

We analyzed 348 individuals belonging to the urban zone of Buenaventura city. The genotypic frequencies found in the population were: E3/E3, 51.1%; E3/E4, 27.3%; E2/E3, 12%; E4/E4, 6%; E2/E4, 3.4%, and E2/E2, 0%; the allelic frequencies were: E3, 70.8%; E4, 21.4%, and E2, 7.7%. The whole population was found in equilibrium of Hardy-Weinberg, with $\chi^2 = 5.2$, and $P = .074$ (Table 1).

Comparing the allelic frequencies between the Buenaventura population and the different populations in Colombia (Table 2), meaningful differences were found for allele E4 in the Waunana, Tule, Yuco, Bogotá, Quindío, Middle-eastern, Valle del Cauca, Barranquilla, and Medellín ($P \leq .0345$). Meaningful differences were also found for the allele E2 in the populations of Quindío, Valle del Cauca, Barranquilla, and Medellin ($P \leq .0232$).

Discussion

There are certain factors converging when you study Buenaventura in the south western Colombian region, the geographic localization, population history, ethnicity and genetic composition of the population which influences in the distribution of *APOE* frequencies. Same as other countries in Latin America, Colombia is a country that presents a great variety of population and ethnicity, clearly visible in 3 groups: Afrodescendant, with a big component of African ancestry; the South American indigenous (*amerindios*) descendants of the ancient and original population of these territories; and the *mestizo* communities, which are the product of a mixture between *amerindios,* Europeans and Africans. Nevertheless, there are variations in relation with the ethnic component which predominates in each region or population in the country,[30] that can be expressed in the differences along the distribution of alleles and genotypes of *APOE,* and thus, in the genetic susceptibility of each one of this populations for developing neurodegenerative and metabolic disorders. In this study, a significant frequency increase was found for allele E4 in the Waunana, Tule, Yuco, Bogotá, Quindío, Middle-eastern, Valle del Cauca, Barranquilla, and Medellín populations, and for the allele E2 in the populations of Quindío, Valle del Cauca, Barranquilla, and Medellin (Table 2). The above can be the

Table 2 – Comparison of the APOE frequencies of different Colombian populations according to the present study (Buenaventura population).

Reference	Region	N	Alleles Observed (%)			Fisher test P-value		
			E2	E3	E4	E2	E3	E4
Current study	Buenaventura	348	7.76	70.83	21.41	-	-	-
17	Providencia	29	8.62	70.71	20.69	0.6104	0.6504	0.5652
	San Basilio	30	5.00	76.67	18.33	0.5900	0.8155	0.3589
	Cauca	25	20.00	60.00	20.00	0.9865	0.1826	0.5445
	Nuqui	30	6.67	73.33	20.00	0.5900	0.6899	0.5283
	Bahía Solano	23	4.34	69.58	26.09	0.4666	0.5368	0.7847
	Chocó	25	20.00	66.00	14.00	0.9865	0.4661	0.3578
	Embera	25	0.00	86.00	14.00	0.1431	0.9877	0.3578
	Waunana	30	0.00	91.67	8.33	0.5900	0.9994	0.0345
	Tule	30	1.67	91.67	6.67	0.3261	0.9994	0.0345
	Ijka	30	1.67	80.00	18.33	0.3093	0.9059	0.3589
	Nukak	20	0.00	62.50	37.50	0.2088	0.3754	0.9478
	Guahibo	26	0.00	80.77	19.23	0.1328	0.9115	0.5046
	Kogí	30	0.00	90.00	10.00	0.0986	0.9966	0.0973
	Coreguaje	28	0.00	58.95	41.05	0.1144	0.1845	0.9883
	Butaregua	21	0.00	90.48	9.52	0.1935	0.9925	0.1474
	Yuco	30	0.00	100.00	0.00	0.0986	1.0000	0.0010
24	Bogotá	538	4.74	86.15	9.11	0.0508	1.0000	0.0000
25	Quindío	500	2.90	95.40	1.70	0.0016	1.0000	0.0000
26	Bogotá	150	5.33	81.33	13.33	1.0000	0.9959	0.0199
27	Middle eastern Colombia	691	4.49	86.83	8.68	0.0232	1.0000	0.0000
28	Valle del Cauca	183	3.00	84.70	12.30	0.0134	0.9999	0.0068
15	Barranquilla	227	1.80	85.00	13.00	0.0010	1.0000	0.0071
11	Medellín	1001	7.50	81.40	11.10	0.4760	1.0000	0.0000
30	Medellín	964	3.9	92.0	4.1	0.0050	1.0000	0.0000

reflection of the difference in the ancestral composition of the population of Buenaventura compared to the populations already mentioned, remembering the high Afrodescendant component of this population (88.5%), in comparison to the other populations whose Afrodescent component does not exceed 40%.[19]

The African population and their descendants are characterized mainly by the high frequency in the allele E4 in comparison with the other racial groups, and the considerable increasing about the allele E2.[7,28,31,32] Considering that the Amerindian population and Afrodescendant population are characterized for present a higher frequency of the alleles E4 and E2, Jaramillo-Correa[17] propose that this equivalency may originate in a bottle neck or the isolation produced by its location between the jungle and the intervened low jungle zone, as also some kind of selection in favor of these alleles as consequence of their basic dependency to hunt and fishing. In Isla de Cascajal, where Buenaventura population originally initiated, in colony time suffered a series of tragic events that could have acted as bottle neck in population (yellow fever, smallpox, fires and earthquakes).[33] The sum of this events, with the founding effect generated, could have changed the allelic frequencies for this gene, coming to what we know actually. The same was reported for the *HbS*, which frequency decreased in Buenaventura's population in a lower number of generations than it was expected only for the selection action.[34]

The reports about the distribution of *APOE* variants shows the high frequencies of E4 in Nigeria, Sub-Saharan Africa, South Africa and the Inuit in Greenland.[8] As a result of the African origin of the Colombian black communities brought to America as slaves during the conquest period and colony period (15th and 16th Centuries), it explain the high frequency of E4 observed in Buenaventura population that shows accurately a genetic similarity with the population of Benin in Africa.[35]

The populations of Providencia Nuquí, San Basilio de Palenque, and Bahía Solano show very similar allelic frequencies as Buenaventura and the reported in west region and central Africa, like Berma (Benin), Fang (Gabon), Bombare (Mali), Nigeria, Peul (Senegal), while Chocó and Cauca are more similar to the frequencies of Malinke (Guinea) and the east coast of Africa, like Merina (Madagascar), Wentworth (South Africa), Tanzania, and Uganda,[36] proving the heterogeneity of places where the people treated as slaves were brought from Africa.[35] Although there are similarities in the *APOE* frequencies, it is not possible declare the origin place of these populations in Africa, because the events of mixture initiated once the slaves were brought from Africa.[36,37]

The local selection, the population structure, and the genetic drift are factors that can bias the results obtained. Other factors as the isolation by distance and the historical mix can also have influenced significantly the distribution of *APOE* polymorphisms in the Afrodescendant population of Buenaventura.

The realization of studies including different phenotypical variables, such as cholesterol level and triglycerides, food types, physical activities, body mass index, etc., will allow to inquire about the role of the natural selection can make on different *APOE* variables in the population. Our results show the utility of the *APOE* locus as an anthropogenic marker of susceptibility to neurodegenerative diseases as Alzheimer, and/or recurrent pathologies by alteration of the lipid

metabolism with especial emphasis in the afrodescendant population.

Conclusions

Buenaventura's population shows evidence on how *APOE* locus is a marker that presents contemporary biological answer to the genetic diversity in the population, this reflects in a major representativeness of alleles E4 and E2 in Afrodescendant populations. Despite of corroboration that the universal marker of *APOE* is the E3, predominating in global populations, the importance of this study highlights an incidence for Buenaventura population, with a 21.4% of the variant E4 and the 7% of the E2, that allows to validate the hypothesis about microevolutive processes in this populations of African origin could considerably alter its frequencies. Finally, it is expected that these results make able to the clinical to get a more real valuation of patients with risk of ailments as Alzheimer and metabolism of cholesterol diseases associated with alleles *APOE* in Colombian population.

Conflict of interests

None declared.

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