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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Evidence of over-dominance for sickle cell trait in a population sample from Buenaventura, Colombia

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Sickle cell disease is a hereditary disease caused by a hemoglobin variant, known as hemoglobin S. This hemoglobin variant has a high frequency in areas where malaria is prevalent, because heterozygous genotype has a protective action against malaria infection. We evaluated evidence of over-dominance or deviation from Hardy- Weinberg equilibrium for the HbS gene in Buenaventura, an afro-descendent population with actual medium endemicity of malaria, located in the Colombian Pacific Coast. 820 healthy individuals were analyzed (from eight months to 80 years old) from Buenaventura, Colombia, and the β -globin locus was genotyped by PCR-RFLP. It was analyzed to establish deviation from Hardy – Weinberg and selection coefficient. Hemoglobin S frequency was 3.1% and the population was found in Hardy- Weinberg equilibrium. However, the selection coefficient for allele S decreased its value of 1 (under 12 years old) to 0.625 (13 to 17 years old), similar to the selection coefficient of allele A (s_1=0.61). Although the total population found in H-W equilibrium, there was a decrease in the coefficient of selection on hemoglobin S in the range of 13 to 17 years old, which is within the age range of the greatest malaria infection in this population. This may be an indication of a selection to the S over-dominance hemoglobin possibly, at least in this age group of the population.

Keys words: Hemoglobin S, Buenaventura, malaria resistant, over-dominance, fitness, selection coefficient.

INTRODUCTION

Sickle cell anemia is an autosome recessive disease, caused by a mutation on beta–globin gene (HBB). This mutation is caused by a transversion (A→T) in the twentieth nucleotide, resulting in the change of glutamic acid to valine in the six codon in the globin (Ingram, 1956). This mutated hemoglobin is also known as S hemoglobin (HbS) and its presence characterizes sickle cell disease (Pauling et al., 1949). When this mutation is in homozygous state (Hb SS), it is called denominated Sickle Cell Anemia (SCA) and when it is in heterozygous state (Hb AS), it is known as sickle cell trait. The individuals with sickle cell anemia present the most severe clinical cases such as: vaso-occlusive crisis, aplastic crisis, hyper hemolytic crisis, splenic sequestration, bacterial infections (in the first months of life), among others. Individuals with sickle cell trait are usually asymptomatic, because of the reasonable amounts of HbA (Ashley-Koch et al., 2000).

The presence of HbS is a well-known example of over-dominance or heterozygous advantage, because AS

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genotype provides protection against malaria. However, natural selection can alter that balance. When the selection favors heterozygote (over-dominance), the gene frequency tends towards an intermediate equilibrium value in which both alleles remain in the population.

Even without mutation, in the cases of over-dominance, the alleles must affect two fitness com-ponents in opposite directions. In sickle cell anemia, the homozygote's fitness reduces through a component serious anemia, while the AA homozygote's fitness reduces due to its susceptibility to malaria and its complications (Falconer and Mackay, 1996).

Haemoglobin disorders contribute to equivalent 3.4% of mortality in children below five years worldwide or 6.4% in Africa (Modell and Darlison, 2008). In 1994, it was reported that life expectancy of those affected by sickle cell disease is not longer than 50 years and 1% children die before they reach the age of 3 (Platt et al., 1994). Nevertheless, this gene is present in Africa and its descendants in America with much higher frequencies than it would be expected due to the compensation between mutation and selection against homozygotes (Haldane, 1949).

Increased homozygous frequencies converge with increased malaria incidence, according to Haldane in 1949; later, Allison (1954) suggested that the presence of these frequencies as well as the maintenance of the sickle-cell polymorphism might have been caused by heterozygous advantage (AS). This is because they have great resistance to *Plasmodium* infection, which is the causative agent of malaria and this is known as "the hypothesis of Haldane" (Haldane, 1949; Allison, 1954; Piel et al., 2010).

Recently Piel et al. (2010) have contributed to the first geographic and quantitative confirmation of the hypothesis of malaria on a global scale. While comparing the frequencies of HbS with worldwide frequencies of malaria endemicity, they found that these relationships are considerably a match in the African continent. However, this relationship is still not clear in America and Asia (Piel et al., 2010). Recently, a study estimated that the global number of neonates affected by HbS in 2010 included 5.476.000 AS and 312.000 SS (Piel et al., 2013). The disease occurs in 1 in every 500 African-American births and 1 in every 1000 to 1400 Hispanic-American births (WHO, 2015).

The maximum predicted frequency in South America to HbS was about 5% (Piel et al., 2013). In Brazil, a HbS frequency of 0.9 to 14% has been reported (Cardoso et al., 2012; De Mello Auricchio et al., 2007; Bandeira et al., 1999), and in Venezuela, up to 7% HbS has been reported (Salazar-Lugo, 2004).

In Colombia, there have been records of hemoglobinpathies frequencies between 2.4 and 14% in different regions (Silva et al., 1998; Bernal et al., 1995; Espinel and Valenzuela, 1991). In the city of Buenaventura, Moyano and Méndez kept records of AS heterozygous prevalence of 7% (Moyano and Méndez, 2005). In 2010, hemoglobinopathies frequencies for a total of 399 infants was revealed, of which 5.8% were heterozygous AC followed by 4.8% heterozygous AS. No presence of homozygous SS was recorded (Bernal et al., 2010).

In the Colombian Pacific Coast, sickle cell disease is an issue of public health. Whether the HbS frequencies are influenced by environmental factors is still unknown. One of those factors can be malaria.

In this study, a healthy population sample was taken from Buenaventura in the Colombian Pacific coast in order to evaluate whether HbS might be favored by natural selection in this population.

MATERIALS AND METHODS

Sample taking in the studied population

Blood samples (4 ml) were collected through venipunture in ethylenedianimetetraacetic acid (EDTA) tubes. A random stratified sampling of 820 people without visible clinical condition participated in the study; they consisted of both genders and with a age range of 8 months to 80 years old (mean = 35.04 years). Samples corresponding to the 12 zones of the City of Buenaventura (Figure 1), an afro-descendent population with actual medium endemicity of malaria, in Colombian Pacific Coast, were included. Visitors or residents who could not prove their original birth in the city were included in this study. Likewise, participants without any kinship relationship with themselves in the same generation (was sampled one only sibling, cousin, grandchild or a family uncle) and members of up to 2 generations per family were allowed.

All participants authorized their entrance to the study by signing an informed consent. This project has the approval of the ethics committee of the Universidad del Valle (Act No. 02-013, 288-012 code).

Molecular diagnosis

DNA was extracted through the method of "salting out" (Miller et al., 1988). DNA amplification was carried out according to the methodology provided by Swee Lay Thein, King's College Hospital in London. The amplification mixture (25 µl of volume): consisted of 50 ng DNA, 1X Taq Buffer, 1.5 mM $MgCl_2$, 0.1 mM of the mixture of the four deoxynucleotides triphosphate (Fermentas, Vilnius, Lithuania), 1.8 uM primer (forward 5'-GGG GGC CTG AAA ATA GTC A-3 'and reverse 5'-GGA AGG AAG AAG ATC AAC AAA GAA GGT C-3') and 0.5 units of Taq DNA polymerase (Bioline, London, UK). The amplification program began with a warm up of 5 min at 94°C for denaturation, 30 cycles continuing as follows: denaturation for 30 s at 94°C, followed by annealing at 62°C for 1 min; finally, an extension at 72°C for 3 min.

The amplified fragment was 571 bp. This was digested with 2 units of restriction enzyme *Dde*I (Promega, Madison, WI, USA) following the manufacturer's directions for use. The digestion was conducted for 5 h at 37°C. Subsequently, the resulting DNA fragments were separated by gel electrophoresis on 8% polyacrylamide and were examined by staining with silver nitrate. Homozygous individuals (SS) affected showed a single band of 308 bp; the heterozygous (AS) three bands, 308, 201 and 107 bp, and homozygous individuals (AA) showed 2 bands of 201 bp and 107 bp.

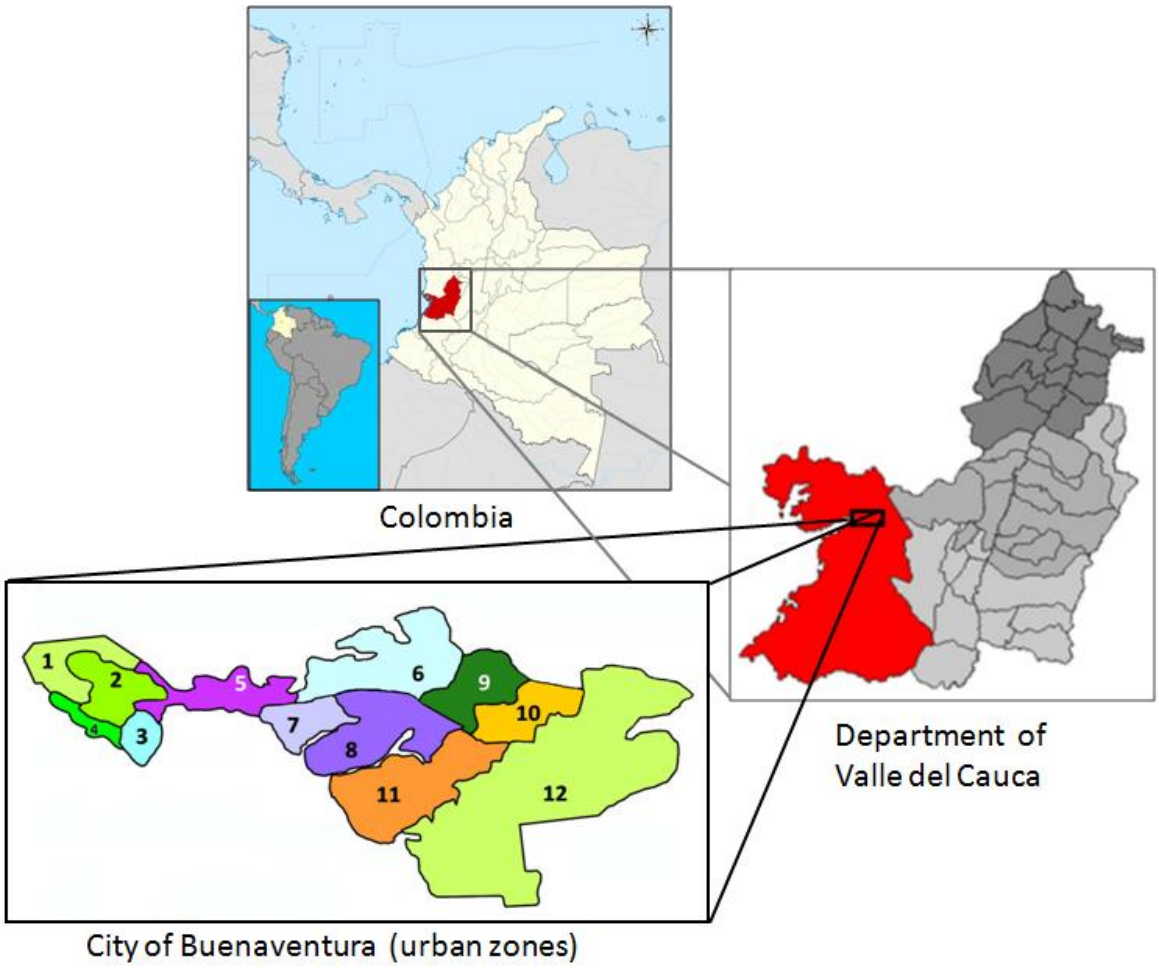

Figure 1. Map of the urban zones of the city of Buenaventura. It shows the Department of Valle del Cauca from the city of Buenaventura, Colombia, and South America country.

Data analysis

The calculation of allele and genotype frequencies, the comparison of the respective genotype frequencies observed and expected (χ^2 test) and the genetic structures among communities (through AMOVA) were conducted with the statistical software GenALEX version 6 (Peakall and Smouse, 2006). The age groups management of data was carried out by the exact test of population differentiation of Raymond and Rousset (Raymond and Rousset, 1995) in the statistical package Arlequin 3.1 (Excoffier et al, 2005). Selection coefficients and fitness for each case were calculated using the formulas proposed by Falconer and Mackay (1996).

RESULTS

A total of 820 individuals were analyzed; there were 214 under-age (18 years old minors) and 606 adults. In the whole sample, 770 (94%) had AA (healthy) genotype, 49 (5.9%) had AS (sickle cell trait) and only one (0.1%) individual had SS (sickle cell anemia), with HbS allele having 3.1% frequency. The needed time to get the observed frequency was calculated from a hypothetical initial frequency of 0.25 using the equation *t= (1/qo-1/qt);* thus the population would need 28.25 generations to reach the observed frequency (Table 1).

The total population was found in Hardy-Weinberg equilibrium, $\chi^2 = 0.656$ with $p = 0.7978$ (Table 1); no significant differences were observed when the population of neonates was compared, as reported by Bernal et al. (2010), with the general population in this research ($p = 0.51070$). In this study, no significant differences were found when both populations were compared; minors and neonates ($p= 0.37830$). Results are not shown.

Before we did the respective analysis of evidence of selection, we divided the groups of children and adults to know if they were homozygote and could be worked as such. The group of children was divided into three subgroups: first, 8 months to 5 years; second, 6 years to 12 years; and third, 13 to 17 years (to embrace the growth phases of childhood and adolescence). Among them, statistically significant differences were found in the

Table 1. Genotype and allelic frequencies and HW equilibrium for the population of 820 individuals in Buenaventura.

Frequency		Genotype				HbS gene frequency	H-W equilibrium
		AA	AS	SS	Total		
Minors	8 months to 5 years old	0.9444	0.0556	0	1.0	0.0278	*p*= 0.7978
	6 to 12 years old	0.9796	0.0204	0	1.0	0.0102	
	13 to 17 years old	0.8548	0.1290	0.0161	1.0	0.0806	
Adults		0.9406	0.0594	0.0000	1.0	0.0297	
Total		0.9390	0.0598	0.0012	1.0	0.031	
N° generations=*t*= (1/*qt**) - (1/*q0**)= 28.2580							

*qt**=0.031; *q0**=0.025.

Table 2. Calculated coefficients selection and fitness for three age subgroups studied.

Case	1) 8 months old to 5 years old vs. adults			2) 6 to 12 years old vs. adults			8 months old to 5 years old vs. 13 to 17 years old		
Genotype	**AA**	**AS**	**SS**	**AA**	**AS**	**SS**	**AA**	**AS**	**SS**
Fitness relative	1.0144	1.0891	0.0000	0.9700	2.9406	0.0000	0.9219	2.3565	0.8871
Fitness relative to SA	0.9314	1.0000	0.0000	0.3299	1.0000	0.0000	0.3897	1.0000	0.3750
Coefficient selection	s_1=0.0686		s_2=1.000	s_1= 0.6701		s_2=1.000	s_1=0.6103		s_2= 0.6250
q value of equilibrium = $s_1/(s_1+s_2)$	**0.0642**			**0.4012**			**0.4940**		

allele frequencies (p = 0.00355) and therefore were evaluated as separate groups (Table 2). In the group of adults, no differences between age ranges were found (p = 0.34115) and they were evaluated as a single group.

Every under-age group was compared with an adult group except the one with children of 13 and 17 years old group. This was compared with the 8 months to 5 years old group to evaluate biological fitness, selection coefficient and *q* value of equilibrium for balanced selection (Table 2). An increase in efficiency for the heterozygous and q increase in equilibrium were observed (q_{eq}), starting with a value of q_{eq}. = 0.0642 for case 1 and ending with a value of 0.49 for case 3. It was also seen how the selection coefficient for allele S decreased its value of 1 (cases 1 and 2) to 0.625 (case 3) similar to the selection coefficient of allele A (s_1=0.61). To validate these operations, we added, for every case, 1 to the absolute genotype frequencies of SS for the minors (Table 2).

The matching between the frequency of genotype AS for the average age of each age range was conducted (Figure 2).The graph shows a heterozygous genotype increase at the average age of 15 years, having its peak at the age of 22 (frequency = 0.0918). From there, there is a decrease in the frequency of the genotype and then a rise from 46 years old (frequency = 0.07) to a frequency of 0.051 at an age of approximately 70 years (Figure 2).

Finally, the genetic structure was observed between the zones (p = 0.001) studied by analysis of molecular variance (AMOVA), where the variation between zones was 3%; while the variation within zones was 97%. The φPT value was 0.030 and Nm = 8.104 (Table 3).

DISCUSSION

The population studied was found in Hardy-Weinberg equilibrium, like that of Bernal's study of neonates in 2010. It was done in three different health institutions of the City of Buenaventura between 2007 and 2008 (Bernal et al., 2010). Unlike them, in this study a recessive homozygous individual was found for HbS (genotype SS) gene.

As the different individuals were evaluated in age range, a possible different selection effect was observed among them. In comparing the group of children between 13 and 17 years old with those between 8 month and 5 years old, a decrease of the coefficient of selection against gene HbS and an increase of q value in equilibrium were observed (Table 2). Although in the adult groups age range there were not found significant differences in HbS frequencies, an increase in heterozygous genotype was seen in particular ages: 18-26 years old and 46-60 years old (Table 3).

The observed decrease in heterozygous individuals and homozygous AA and SS during early ages could be because these are affected by children's illnesses. Buenaventura has a substantial rate of child mortality. According to 2004 data, death was observed in 36/1000

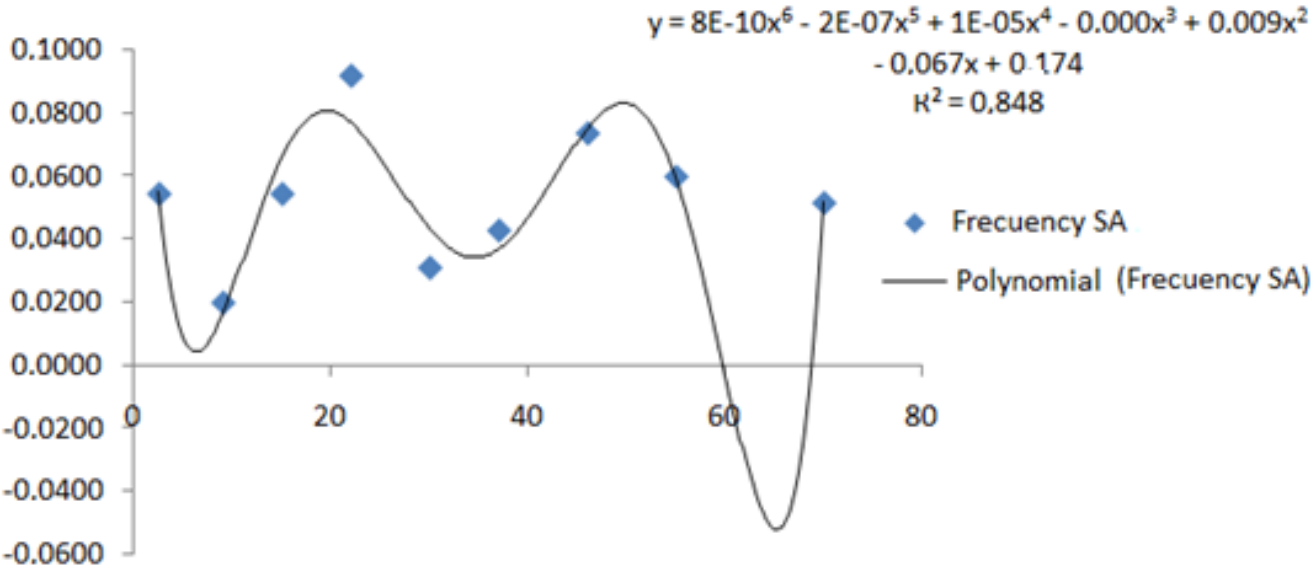

Figure 2. Graphic of the relationship between the average age (X) and genotype SA frequency (Y). Polynomial trend line type of order 6 show relationship between these two variables.

Table 3. AMOVA and genetic structure found for the 12 zones of Buenaventura city.

Level	df	Sum of squares	Mean squares	Variance	%
Among zones	11	1.988	0.181	0.002	3
Within zones	808	47.840	0.059	0.059	97
Total	819	49.828		0.061	100
ϕ_{PT} =0.030			***P*= 0.001**		***Nm* =8.104**

children under 5 years old and in less than 1 year old (31. 4/1000). The leading causes of deaths in infants are: respiratory disorders (28.14%), congenital malformations (10.18%), other conditions originating from the perinatal period (9.37%), and acute respiratory infections (22%) (Diagnóstico socio-económico de Buenaventura, 2015). Therefore, under these circumstances, healthy heterozygous and homozygous children are equally susceptible; except when cultural customs of preventing mosquito like using of mosquito nets for little children are used to reduce the incidence of malaria (Méndez and Carrasquilla, 1995). This shows the HbS allele can reach its highest value of equilibrium ($_q$= 0.494) in 13 to 17 years old group and its lowest value in 8 months to five years old (*q*=0.064) group. This HbS allele equilibrium value during adolescence can be related with an acquired immunity when individuals begin to manifest AS. In malaria zones, there are 9 years old AS children with a minor risk of developing symptomatic malaria than 2 years old children. This suggests an acquired defense mechanism (Williams et al., 2005).

Moreover, an increase in frequency of heterozygote children is observed. They reach a frequency between 7.3 to 9.0% (Figure 1). These peaks suit the age range where malaria is lethal in Buenaventura, which is between 15 to 60 years old (Méndez and Carrasquilla, 1995). The frequency of heterozygous AS decreases in the range of 30 to about 40 years old. This may be due to the acquired resistance to malaria in people after repeated episodes of the infection. This immunity depends on the genotype acquired not present in the beta-globin. Therefore, individuals between 30 and 40 years old of AA genotype have equal probability of survival than heterozygous AS (Gong et al., 2012), implying a higher proportion of AA in this age range. This phenomenon would also explain why there was no variation in the selection of coefficient in adults.

In the infant population of Buenaventura, especially those older than 6 years, there is evidence of selection effect (Table 2). From 6 to 12 years old (case 2) group, the effect on the A allele is remarkable, since there is a significant increase in the selection of this coefficient with respect to that in 8 months to 5 years old group (case 1). This also is reflected in the q equilibrium, again according to case 1. Likewise, in 13-17 years old (case 3) group, there is an evidence of selection action but this time on the

S allele, where there is a decrease in the allele coefficient of selection. The selection acts in a less severe way on this range of age allele (Table 2).

Although effects of natural selection were found in the children population, the total sample was found in Hardy-Weinberg equilibrium. These results can be an indication of the presence of a founder effect and / or bottleneck in the population. Buenaventura town, during late nineteenth and early twentieth centuries, suffered several catastrophic events: yellow fever and smallpox wiped out nearly a fifth of the population, a series of fires destroyed the few existing buildings, and in 1931 another fire damaged important areas of the city (Pérez, 2007). These events made the population to go through several bottlenecks, where drift prevails as evolutionary force. Under these conditions, selection effects will be superimposed on the drift, in addition, because the drift's effects were permanent in the population after the bottlenecks (Luzzato, 2012).

The action of selection in favor of HbS is not observed in the whole population. In fact, the action of selection was reflected in only one segment of the population and not in all of it. Moreover, the frequency of HbS decreased faster in the population than expected: if the selection was only taken into account, the time required to achieve the current rate would be 28 generations (706 years) (Table 1). This time period is greater than the period of time since the creation of the first settlements in Buenaventura (~ 470 years), which leaves genetic drift as a most likely mechanism to explain the current frequency of HbS in Buenaventura.

Indeed, Buenaventura recently lived or might still be living a small phenomenon founder effect. The genetic structure is observed among the zones of the city (Table 3). This is a clear indication of the recent formation of these: until just 60 years ago, Buenaventura was concentrated on the Cascajal Island and the "mainland region" was gradually colonized.

These new subpopulations could take subsamples of the genetic diversity of the population "mother" (the town located on the Cascajal Island), a phenomenon that would add to the diversity which people bring from different regions of Colombia's Pacific Coast; those who have come and are still coming to Buenaventura and those who are located in this island.

There are clear indications that there are dynamics of change in the frequency of HbS in the town of Buenaventura depending on the frequency of malaria infection. This could indicate this module disease and can be a factor of pressure on the HbS in Buenaventura. However, a confirmation data of a case of over-dominance could be tested if there are significant differences in density of the pathogen between heterozygote (AS) and AA people. A quantitative analysis of *Plasmodium falciparum* using molecular method like q-PCR or reverse-transcriptase PCR rounds up our results. Now, knowing the condition of over-dominance for gene HbS in Buenaventura, the risk for the occurrence of new cases of sickle anemia in the population (as well as the most susceptible age groups) is clearer, as long as the pressure of selection is maintained for heterozygous individuals.

Finally, although AS individuals are usually asymptomatic, it is important to give them genetic counseling and to educate them about their status in order not to give birth to homozygous children and to avoid psychosocial issues such as stigmatization and misunderstanding because of their status as carriers.

Conflicts of interest

The authors have not declared any conflict of interest.

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REFERENCES

Allison AC (1954). Protection afforded by sickle cell trait against subtertian malarial infection. Br. Med. J. 1 (4857): 290–294. http://dx.doi.org/10.1136/bmj.1.4857.290

Ashley-Koch A, Yang Q, Olney RS (2000). Sickle Hemoglobin (HbS) Allele and Sickle Cell Disease: A HuGE review. Am. J. Epidemiol., 151(9): 839-845. http://dx.doi.org/10.1093/oxfordjournals.aje.a010288

Bandeira FM, Leal MC, Souza RR, Furtado VC, Gomes YM, Marques NM (1999). Hemoglobin "S" positive newborn detected by cord blood and its characteristics. J. Pediatr. (Rio J), 75(3):167-171. http://dx.doi.org/10.2223/JPED.292

Bernal MP, Giraldo A, Bermúdez A J, Moreno E (1995). Estudio de la frecuencia de hemoglobinopatías en las islas de San Andrés y Providencia, Colombia. Biomédica 15(1): 5-9.
http://dx.doi.org/10.7705/biomedica.v15i1.850

Bernal M, Collazos A, Bonilla R, Tascón E (2010). Determination of the prevalence of hemoglobins S, C, D, and G in neonates from Buenaventura, Colomb. Med. 4(2): 141-147.

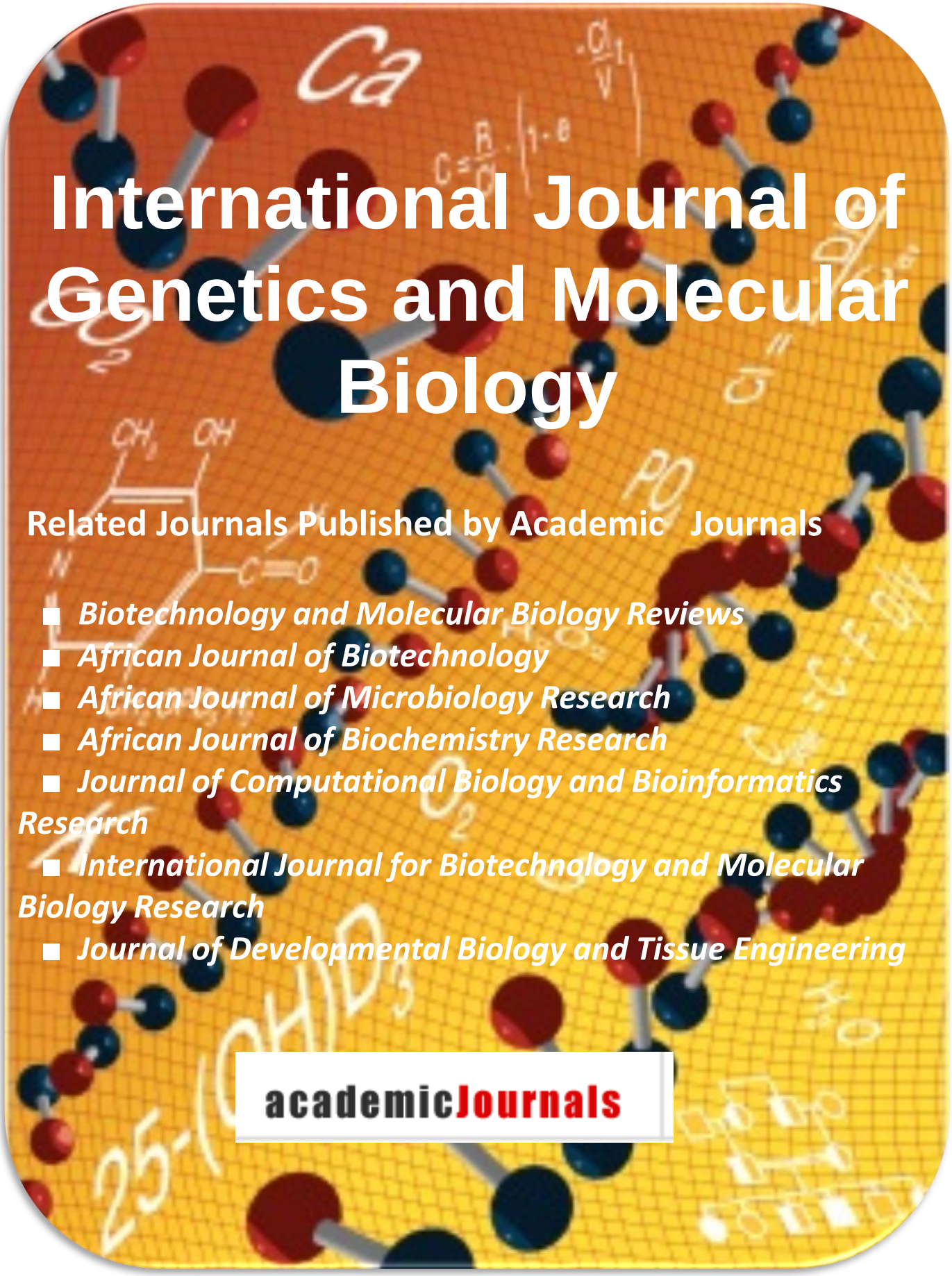
Cardoso GL, Takanashi SY, Guerreiro JF (2012). Inherited hemoglobin disorders in an Afro-Amazonian community: Saracura. Genet. Mol. Biol. 35(3): 553-556. http://dx.doi.org/10.1590/S1415-47572012005000041

De Mello AMT, Vicente JP, Meyer D, Mingroni-Netto RC (2007). Frequency and origins of hemoglobin S mutation in African-derived Brazilian populations. Hum. Biol. 79(6):667-77.

Espinel AE, Valenzuela N (1991). Adaptaciones genéticas a la malaria en poblaciones afroaborígenes del pacífico colombiano. Rev. Antropol. 7:119-129. ISSN: 0124-485X

Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An

integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1: 47-50.
Falconer DS, Mackay TF (1996). Introduction to Quantitative Genetics. 4th edición. Pearson Prentice Hall. Gobernación del Valle de Cauca. Diagnóstico socio-económico de Buenaventura http://www.valledelcauca.gov.co/planeacion/descargar.php?id=83 [access on January 25 2015].
Gong L, Maiteki-Sebuguzi C, Rosenthal PJ, Hubbard AE, Drakeley CJ, Grant D, Greenhouse B. (2012). Evidence for both innate and acquired mechanisms of protection from Plasmodium falciparum in children with sickle cell trait. Blood 119(16):3808-3814. http://dx.doi.org/10.1182/blood-2011-08-371062
Haldane JBS. (1949).The rate of mutation of human genes. Hereditas 35:267-273. doi: 10.1182/blood-2011-08-371062. http://dx.doi.org/10.1182/blood-2011-08-371062
Ingram V (1956) A specific chemical difference between the globins of normal human and sickle cell anemia haemogliobin. Nat. 178(4537): 792-94. http://dx.doi.org/10.1038/178792a0
Luzzato L (2012).Sickle Cell Anaemia and Malaria. Mediterr. J. Hematol. Mediterr. J. Hematol. Infect Dis. 4(1): e2012065. http://dx.doi.org/10.4084/mjhid.2012.065
Méndez F, Carrasquilla G (1995). Epidemiologia de la malaria en el área urbana de Buenaventura: análisis de la ocurrencia en el periodo 1987-1993. Colomb. Med. 26(3): 77-85.
Miller S, Dykes D, Polesky H (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3): 1215. http://dx.doi.org/10.1093/nar/16.3.1215
Modell B, Darlison M (2008). Global epidemiology of haemoglobin disorders and derived service indicators. Bull WHO, 86(6):480-487. http://dx.doi.org/10.2471/BLT.06.036673
Moyano M, Méndez F (2005). Defectos eritrocíticos y densidad de la parasitemia en pacientes con malaria por Plasmodium falciparum en Buenaventura. Rev. Panam Salud Pública 18(1):25-32. http://dx.doi.org/10.1590/S1020-49892005000600005
Pauling L, Itano H.A, Singer J, Wells IC (1949). Sickle cell anemia, a molecular disease. Sci. 110(2865): 543-548. http://dx.doi.org/10.1126/science.110.2865.543
Peakall R, Smouse PE (2006). GENALEX 6: Genetic Analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288-295. http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x
Pérez GJ (2007). Historia, geografía y puerto como determinantes de la situación social de Buenaventura. En: Documentos de trabajo sobre economía regional No 91. Banco de la República. Centro de estudios económicos regionales CEER, Cartagena.
Piel FB, Patil A, Howes R, Nyangiri O, Gething PW, Williams T. Weatherall DJ, Hay SI (2010). Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. Nature Com. 2010; 1-104. http://dx.doi.org/10.1038/ncomms1104
Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, Temperley WH, Williams TN, Weatherall DJ, Hay SI (2013). Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet 381(9861):142-51. doi: 10.1016/S0140-6736(12)61229-X. http://dx.doi.org/10.1016/S0140-6736(12)61229-X
Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP (1994). Mortality in sickle cell disease--life expectancy and risk factors for early death. New Eng. J. Med. 330(23): 1639-1644. http://dx.doi.org/10.1056/NEJM199406093302303
Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49(6):1280-1283. http://dx.doi.org/10.2307/2410454
Silva JR, Malambo D, Silva DF, Fals E, Fals O, Rey J (1998). Tamizaje de hemoglobinopatías en una muestra de la población infantil de Cartagena. Pediatría 33(2):86-89.
Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, Snow RW, Marsh K (2005). Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. J. Infect. Dis., 192(1): 178-86 PMID:15942909. http://dx.doi.org/10.1086/430744
World Health Organization (2015). Genes and Human research. http://www.who.int/genomics/public/geneticdiseases/en/index2.html#SCA [access on January 25 2015].



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