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Genetic Variants of Duffy and Hemoglobin S Genes in an Afrodescendent Population from Columbia

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Abstract

Malaria is an endemic disease in a large part of Colombia, and the city of Buenaventura reports one of the highest malaria infection rates. Some genetic variants confer resistance to malaria, such as the heterozygote for hemoglobin S (HbS) and the homozygous variant FYB^{ES}/FYB^{ES} of the Duffy gene. The aim of this work was the molecular characterization of these genes in an afrodescendent population from the urban area of Buenaventura. A total of 819 individuals from a stratified random sampling in each of the 12 communities of this city were analysed. Molecular analysis was performed using PCR-RFLP, and data analysis was performed using the Arlequin 3.5, SPSS 20 and R 3.4.1 programs. Frequencies of 3.1% and 72.2% were obtained for the S and FYB^{ES} alleles, respectively, and the values were 6.1% and 55% to AS and FYB^{ES}/FYB^{ES} genotypes respectively. The highest proportion of these resistance genotype (genotypic combination AA*FYB^{ES}/FYB^{ES}) were showed for the group of 13 to 27 years with (8.2%) and the communities 1 and 3 (18% and 10.3%, respectively). Therefore, it would be pertinent to consider these communities and age groups when performing epidemiological studies and preventive and health care campaigns on malaria in the urban areas of the city of Buenaventura.

Malaria is one of the leading causes of death worldwide and has been suggested to be the most potent selection factor in humans (Kwiatkowski 2005). In addition, it is an endemic disease in a large part of Colombia, especially in Buenaventura, a municipality of Valle del Cauca. This municipality reports one of the highest malaria infection rates, as well as the highest number of malaria-related deaths.

Some genetic variants confer resistance to malaria, as is the case for the haemoglobin S (HbS) heterozygote and the FYB^{ES}/FYB^{ES} Duffy gene homozygote. In regions with Afro-descendant populations, where malaria is endemic, high frequencies of the S allele have been found for HbS, represented primarily as a high frequency of heterozygous individuals (AS), which confers the advantage of showing greater resistance to infection by *Plasmodium*, the causal agent of malaria. Analogously, homozygosis for the genotype FYB^{ES}/FYB^{ES} confers resistance to *P. vivax* malaria, and the FYB^{ES} allele has been found in a greater proportion in the Afro-descendant population and in regions endemic to malaria (Eridani 2011; Howes et al. 2011; Luzzatto 2012; Zimmerman et al. 2013).

In Colombia, hereditary genetic diseases, such as sickle cell disease, prevail, and in southwestern Colombia, the population is susceptible to high frequencies of the HbS and Duffy-null genes. Specifically, the genotypic prevalences of the HbS and Duffy genes have been established, primarily in coastal populations, such as Chocó, the rural area of Buenaventura, Córdoba, Tierra Alta and Cartagena (Bernal et al. 1995; Silva et al. 1998; Moyano and Méndez 2005; Bernal et al. 2010; Alvear et al. 2012; Gonzalez et al. 2012; Vallejo et al. 2015). However, no population-based studies have examined the genotypic frequencies of both genes or whether the combination of the genotypes, that confer resistance, is primarily found in a specific age group or in specific areas of the population.

The goal of this study is the molecular characterization of the Duffy and HbS genes in the urban population of the city of Buenaventura, an endemic region for malaria in Colombia. The allelic and genotypic frequencies for the Duffy and HbS genes were established, and the combinations of these two variants were determined in each of the 12 communities of urban Buenaventura to determining the level of protection that these variants could confer against malaria. Additionally, the allelic and genotypic frequencies for these two genes were compared with those reported for other Colombian populations.

Materials and Methods

Sample Collection and Type of Study

A cross-sectional study was performed using stratified random sampling, where each "stratum" corresponded to one of the 12 communities of the city of Buenaventura, a municipality of Valle del Cauca (Figure 1). DNA samples from 819 healthy individuals, both males and females, were analysed, of which 214 were underaged and 605 were adults. The sampling did not include foreigners, visitors, or inhabitants who could not demonstrate origins from the city or could not present a birth certificate. Unrelated individuals were chosen (not related to each other in the same generation: only one sibling, cousin, grandson, and/or uncle per family), and only members of a maximum of 2 generations per family were allowed. The samples were stored at -20°C in the Human Molecular Genetics Laboratory of the *Universidad del Valle*, where they were processed. This research was approved by the Human and Animal Ethics Committee of this university (Certificate No. 02-013 and 013-017).

Molecular Diagnosis

DNA was extracted through the "salting out" method (Miller et al. 1988). The DNA amplification of the region corresponding to the HbS gene was conducted according to the methodology provided by Swee Lay Thein, King's College Hospital in London: 50 ng DNA, 1X Taq Buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 1.8 uM primers (forward 5'-GGG CTG GGC ATA AAA GTC A-3 'and reverse 5'-AGG GGA AAG AAA ACA TCA AGG GTC -3') and 0.5 units of Taq DNA polymerase. The 571 bp frament fragment was treated with with 2 units of restriction enzyme *DdeI* and the generated fragments were visualized on 8% polyacrylamide gels. The products of this digestion were 308 bp for genotype SS; 308, 201 and 107 bp for phenotype AS; and of 201 bp and 107 bp for phenotype AA.

For the "GATA" promoter region of the Duffy gene, the amplification mix consisted of the following: 50 ng DNA, Buffer *Taq* 1X, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.3 µM of each primer (*forward*: 5'-GGG CTG GGC ATA AAA GTC A-3' and *reverse*: 5'- AGG GGA AAG AAA ACA TCA AGG GTC -3'), and one unit of *Taq* DNA polymerase. The 392 bp fragment was treated with three units of the restriction enzyme *StyI*, and the generated fragments were visualized on 8% polyacrylamide gels. The combination of 205, 110 and 65 bp fragments corresponded to Duffy negative (Fy-), while the combination of 205, 110, and 77 bp bands corresponded to Duffy-positive (Fy+).

For the Duffy antigen receptor for chemokines (DARC) coding region of the Duffy gene, the amplification mix consisted of the following: 50 ng DNA, Buffer Taq 1X, 1.5 mM $MgCl_2$, 0.1 mM of each of the four deoxynucleotide triphosphates mixes, 0.5 µM of each primer (*forward*: 5'-AACTGAGAACTCAAGTCAGC-3' and *reverse*: 5'-ATGAAGAAGGGCAGTGCAGAGT-3') and one unit of *Taq* DNA polymerase. Subsequently, the 159 bp fragment was treated with one unit of the restriction enzyme *Ban-I.* The products of this digestion were 159 bp for

phenotype Fy(a-b+), 86 and 73 bp for phenotype Fy(a+b-), and 159, 86, and 73 bp for phenotype Fy(a+b+).

Data Analysis

Descriptive Statistics, Allelic Frequencies and Differentiation of Subgroups by Age. The study variables are described in Table 1. To evaluate subgroups by age, allele frequencies were calculated and the Raymond and Rousset exact test for population differentiation (Raymond and Rousset 1995) was performed, using the statistical package Arlequin version 3.5.2.2 (Excoffier and Lischer 2010).

The age data was not available for 25 of the 819 sampled subjects, therefore they were excluded from the analysis that required this information. ·A value of 0.05 was considered to be the maximum accepted value for a type I error.

The variable called "Protection" was created in this study in order to summarize and compile, for epidemiological purposes, the representative genotypic combination for both genotypes of malaria protection. The "Double complete" category is represented by those individuals who have both, AS and FYB^{ES}/FYB^{ES}, resistance genotypes, which confer resistance to falciparum and vivax malaria, respectively (combination 7, table 1). The "Double partial" category contains individuals who have complete protection against falciparum malaria and a heterozygous Duffy-null genotype (combinations 11 or 12, table 1). The "single complete" category is represented by those individuals that only have one resistance genotype (either AS or FYB^{ES}/FYB^{ES}), and a genotype without these protection alleles (combinations 1, 8, 9, 10 or 13). The "single partial" category has individuals that are only heterozygous for FYB^{ES} (combinations

5 or 6, table 1). Finally, the category called “None" is represented by individuals who do not carry any allele of resistance AS or FYB^{ES} (combinations 2, 3 or 4, table 1).

Independence Test, Analysis of Variance, Regression and Correlation. Independence between the variables was measured by Fisher’s exact test (1935), and a multinomial or multi-ordinal regression analysis was also performed using the statistical software R, version 3.4.1 (Core Team 2017). The comparison of deviance between communes was calculated using the mean squares, obtained from a generalized linear model with a distribution of “quasipoisson” errors.

Bayesian Analyses for the Estimations of Prevalences. The *a priori* distribution was elicited using a uniform probability distribution, which was combined with data from the field, obtaining the posterior distribution of prevalences. With the latter, an indicator, expected value, or estimator of the prevalence of the variable "protection" was obtained.

Population Differentiation and Homogeneity Test. The allelic and genotypic frequencies of the study population were compared with reports from different Colombian populations using the Raymond and Russet test (Raymond and Rousset 1995),(Raymond and Rousset 1995)¹⁵ complemented with the χ^2 homogeneity test, using the Arlequin v3.5.2.2 statistical packages (Excoffier and Lischer 2010) and SPSS v20 (Corp IBM Released 2011). There were no studies reporting the combined frequencies of the Duffy and HbS genes; therefore, these genes were evaluated separately for the studied populations. For the Duffy gene, the populations of rural Buenaventura (Valle del Cauca), Tumaco (Nariño) and Tierra Alta (Córdoba) (Vallejo et al. 2015), as well as a population from La Italia (Chocó) (Gonzalez et al. 2012), were compared. For the HbS gene, two Buenaventura (Valle) populations (Moyano and Méndez 2005; Bernal et al. 2010), a population from San Andrés y Providencia (Bernal et al.

1995), a population from Cali (Valle) (Satizabal Soto et al. 2013) and two populations from Cartagena (Bolívar) (Silva et al. 1998; Alvear et al. 2012) were compared.

Results

Allelic and Genotypic Frequencies and Differentiation by Age Ranges

A total of 819 individuals from 12 communities of the urban areas of Buenaventura, Colombia, were analysed (Table 1): 214 were underaged and 605 were adults, with an age range from 8 months to 93 years (mean = 35 years, SD = 20 years).

For the HbS gene, 769 carried genotype AA (normal haemoglobin), 49 genotype SA (carriers of the sickle trait) and only one individual carried genotype SS (homozygous anaemic). Thus, the frequency for allele S was 3.1% and for allele A was 96.9%; the AA genotypic frequency was 94%, SA was 5.9% and SS was 0.1% (Table 2).

For the Duffy gene, 450 individuals (55%) carried the homozygous mutation for Duffy negative (genotype FYB^{ES}/FYB^{ES}), which was the most frequent genotype, followed by genotypes containing the alleles FYA/FYB^{ES} (18.6%) and FYB/FYB^{ES} (14.9%). The FYA/FYB genotype represented 6 % of the population, while the least frequent genotypes were FYA/FYA and FYB/FYB, corresponding to 3% and 2.5% of the samples, respectively. Thus, the allelic frequency for FYB^{ES} was 72%, while the FYA and FYB alleles had allelic frequencies of 15% and 13%, respectively (Table 3).

When analysing the genotypes for the HbS and Duffy genes among individuals in different age ranges (8 months to 12 years, 13 to 26 years, and 27 to 93 years), significant differences were found for both genes together ($p = 0.010$) and separately for the HbS gene ($p = 0.001$), but not for the Duffy gene ($p = 0.423$). The group aged 13 to 26 years showed significant differences

compared to the groups aged 8 months to 6 years ($p = 0.016$) and 27 to 93 years ($p = 0.012$). These age groups were considered in the different tests.

Independence Test, Multiple Regression, Analysis of Variance and Prevalences

The Fisher's independence tests were significant ($p \leq 0.05$) for the variables "Community" and "Age group" (Table 1) for all variables, except for the variable "Duffy mutation", which showed no dependence on the variable "Age" but was dependent on the variable "Community" (Table 3). The results of the multinomial regression (and multinomial regression for the response variable "Protection") showed that the variable "Community" also influenced all the study response variables (Table 3), and the variable "Age group" influenced the variables "HbS mutation" and "Protection". Regarding the variable "Gender", no significance was found for the response variables for either Fisher's test or the multiple regressions.

The analysis of deviance for variable protection, in relation to the Communities, showed that these differences were specific between communities 3 vs 6 and 12, and community 1 vs 6, 7 and 12. Regarding age groups, according to the demographic test of Raymond and Russet (Raymond and Rousset 1995), the analysis of variance showed that the group aged 13 to 26 years was significantly different from the group aged 8 months to 12 years and from the group aged 27 to 93 years ($p = 0.01$).

The three age groups showed that the category "single complete" protection was predominant, followed by "single partial" protection. For the group aged 13 to 26 years, the category "double complete" protection was as prevalent as the category "no protection", with 8.2% for both, unlike the other age ranges, where the variable "double complete" ranked fourth among prevalences out of the 5 pre-established categories (Figure 2). Finally, "double-partial"

protection was the least prevalent in all categories and was primarily represented in the group aged 13 to 26 years, at 5.41% (Figure 2).

All the communities had the highest prevalence of individuals with "single protection", showing frequencies between 40.3% and 63.5%, followed by the category "single partial" protection (23.4 to 43.1%). The communities with highest prevalence of "double complete" protection were 1 and 3, with 18% and 10.3%, respectively. In turn, the category 'no protection' showed a higher prevalence in communities 7, 6, 12 and 10, with 26.8%, 19.4%, 17.7% and 13.6% respectively. All protection categories were present in the 12 communities except for "double complete" protection, which was not represented in community 4; in turn, "double partial" protection had no representation in communities 2, 5, 6 and 11 (Figure 3, Table 4).

Comparison with Other Colombian Populations

The population of this study showed significant differences in terms of genotypic frequency distributions for the Duffy gene when compared to the populations of rural Buenaventura ($p = 0.0001$), Tierra Alta ($p < 0.0001$), and La Italia (Chocó). However, when the population from this study was compared to the population of Tumaco, the difference was not significant ($p \geq 0.5751$). For the HbS gene, the population of this study showed marked differences in genotypic frequencies when compared to the population of Cali (Valle), although this was not the case for the other Colombian populations, such as San Andrés and Providencia, Cartagena and Buenaventura.

Discussion

The variables of age and community appear to be related or to influence the patterns of mutations for the HbS and Duffy genes, as well as the combination of these two genes in the variable "protection". Regarding age, the group aged 13 to 26 years showed a different distribution in terms of the category of variable protection when compared to the other age groups; both genotypes that confer resistance to malarial infection (AS and FYB^{ES}/FYB^{ES}) occur at their highest frequencies in this age group (Figure 2), and this age group contains a greater proportion of individuals who fall within the categories of "double complete" protection and "double partial" protection and a decrease in the proportion of individuals who fall within the category "no protection". Therefore, this group shows a higher proportion of genetic mutations that could confer greater resistance to malarial infection compared to other age groups. In turn, the communities with the highest prevalence of individuals with protective genotype were 1 and 3. In contrast, the communities with the highest prevalence of non-protective genotypes were 6, 7 and 12. Valle del Cauca, particularly Buenaventura, shows the greatest incidence of malaria cases, primarily in individuals from 15 to 45 years of age, but mortality predominates in those over 60 and under 5 (Fernández et al. 2009). Several studies from endemic malaria areas in Colombia show that infection rates increase with age, with the highest rates occurring in adolescents and young adults and beginning to decrease in older adults, who show the lowest rates (Mendez and Carrasquilla 1995; Mendez et al. 2000; Fernández et al. 2009). This pattern has been associated with the behavioural factors of these population groups, such as higher exposure to work activities in young people and adults, which, in many cases, includes moving within the rural area, increasing exposure to mosquito bites. In turn, young children and older adults are generally less exposed to mosquito bites and show lower infection frequencies (Mendez et al. 2000; Ortega et al. 2015). However, despite the fact that adolescents and young

adults feature the highest infection rates, they apparently show higher survival rates in response to this infection compared to other age groups (Fernández et al. 2009), especially when compared to children under 5 years of age, who represent approximately 70% of malaria-related deaths worldwide (WHO 2017). These observations support the hypothesis that young people and adults show reduced mortality due to the studied genetic mutations of resistance, additionally, adults may have acquired immunity due to episodes of malaria infection presented throughout their life (Doolan et al. 2009; Pinkevych et al. 2012). The resistance provided by the AS genotype is focused on how the organism copes with the disease. AS individuals may become infected by malaria, but their erythrocytes are less prone to infection than those from individuals without the trait; they show lower incidences of cerebral malaria and malaria with severe anaemia, and, in consequence, heterozygous individuals rarely die of malaria (Olumese et al. 1997; Luzzatto 2012). In turn, although there are reports of Duffy negative individuals infected with *Plasmodium vivax* in some regions of the world (Ménard et al. 2010; Russo et al. 2017), many of these individuals can act in a manner similar to collective immunity to reduce transmission and, consequently, protect Duffy positive individuals from *vivax malaria* (Zimmerman et al. 2013). In this sense, having "resistant" genotypes do not confer immunity to the disease; however, the collective protection or immunity provided within a population with high frequencies of these genotypes helps the organism cope with the development of the disease. Studies are needed to determine whether *P. vivax* exclusively infects Duffy negative individuals in Buenaventura because, in reports related to the mestizo, Afro and indigenous population of Chocó, *P. vivax* was found only in Duffy positive individuals (Gonzalez et al. 2012).

Reports from 1987 to 1993 have shown that the communities with the lowest rates of malarial morbidity have been 1, 2, 3, 4 and 5 (Mendez and Carrasquilla 1995), while 1, 4, and 5 showed the lowest infection rates in 2016 and 2017 (Perea 2016; Perea 2017). These communities are located in the insular area of urban Buenaventura, the oldest area of the city, originally known as Cascajal Island. (Pérez V. 2007). Due to their ages, these communities have had more time to adapt to the disease through genotypes that are resistant to malaria, and those communities with higher percentages of individuals with "double complete" protection genotypes were found in that insular zone (1 and 3, with 18% and 10.3%, respectively) (Figure 3). In turn, the communities with higher prevalence of "unprotected" individuals were 6, 7, 10 and 12, with prevalences of 19.4%, 26.8%, 17.7% and 13.6% respectively (Figure 3), which the deviance analysis showed were significantly different from those of the other communities. Between 1987 and 1993, urban Buenaventura reported the highest malarial morbidities in communities 9, 10 and 12 (Moyano and Méndez 2005). Between 2016 and 2017, these three communities, in addition to community 11, remained among the most affected (Perea 2016; Perea 2017); contrary to communities with the lowest infection rates, which correspond geographically with the continental area of urban Buenaventura, this area has been the most affected by malarial infection for 30 years or more, especially community 12, which represents approximately 20% of the urban population. Seventeen years ago, this vulnerability was attributed to a high degree of unmet basic needs, high forest density, and natural mosquito outbreaks (Olano et al. 1997). Historically, the health problems of the city of Buenaventura have been quite critical due to the deficiency in the provision of health services and the ability to respond to the demands of the population. Approximately 49.1% of the population does not have health insurance (CDB, 2016), and although the protection and prevention strategies

implemented by the community have decreased malaria infection and mortality rates (Alvarado et al. 2006), statistical data show that these communities were and will remain among the most vulnerable in terms of malarial infection, not only because of human and social factors but also because genetically, as found in this study, they show the lowest genotypic frequencies for protection, both for vivax and falciparum malaria (Perea 2016; Perea 2017). When performing epidemiological studies and prevention and health care campaigns related to malaria, it would be appropriate to prioritize these communities in urban Buenaventura.

According to the distribution of genotypic frequencies, the urban and rural populations are not similar, at least regarding the Duffy system. The rural population of Buenaventura, as reported by Vallejo and collaborators (Vallejo et al. 2015), is composed of the localities of Punta Soldado, Zacarias and La Delfina, with the latter showing the highest frequency of Duffy positive individuals (explained perhaps by the high indigenous component of the area), thus changing the distribution of frequencies, with increases in the FYA allele frequency and the frequency of the FYA/FYA genotype among the population. Similar cases occurred with the populations of Tierra Alta (Córdoba) and La Italia (Chocó), which showed that the genotypic frequencies for the Duffy gene were completely different from those of other populations, with significantly high frequencies of Duffy positive individuals, for both the FYA and FYB alleles, which was attributed to a high degree of mixing with the indigenous population and the reduced migration patterns of Afro-descendants (Vallejo et al. 2015). For the HbS gene, the genotypic frequencies of the studied Buenaventura population were not significantly different from those of other populations, except for the population of Cali. (Satizabal Soto et al. 2013) This result is consistent with the fact that, of all the reported populations, Cali features the lowest percentage

of Afro-descendants (approximately 40%) and, therefore, a lower HbS allele frequency than others.

In conclusion, adolescents and young adults from urban Buenaventura show the highest percentage of genotypic resistance to malaria, as do the communities 1 and 3, which are located in the insular and oldest area of the island. In turn, the remaining age groups, along with communities 6, 7, 10 and 12, which correspond to the continental zone of the island, could be more susceptible to malaria infection because of their low frequency of resistant genotypes. Finally, regarding the Duffy gene, the urban population of Buenaventura cannot be completely equated with the rural population due to the differences in allelic and genotypic frequencies, a fact largely associated with the extensive proportion of the indigenous population living in the rural area.

The present findings have important applications in the design of epidemiological studies, as well as in preventive and health care campaigns against malaria in urban Buenaventura.

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Table 1. Variables of Study

Variable	Categories or values	Type of variable	Scale of measure	Method of measure
Sex	1= female 2= male	Qualitative	Nominal	Poll
Age	1= 8 months to 12 years 2=13 to 26 years 3=27 to 93 years	Continous quantitative	Ratio/Ordinal[a]	Poll
Community	1= Community 1 2= Community 2 (…) 12= Community 12	Qualitative	Nominal	Poll
HbS Mutation	1=AA 2=AS 3=SS	Discreet quantitative	Ratio / Nominal[a]	PCR-RFLP and later band counting
Duffy Mutation	1= FYB^{ES}/FYB^{ES} 2= FYA/FYA 3= FYB/FYB 4= FYA/FYB 5= FYA/FYB^{ES} 6= FYB/FYB^{ES}	Discreet quantitative	Ratio / Nominal[a]	PCR-RFLP and later band counting
Combination HbS and Duffy	1= AA + FYB^{ES}/FYB^{ES} 2= AA + FYA/FYA 3= AA + FYB/FYB 4= AA + FYA/FYB 5= AA + FYA/FYB^{ES} 6= AA + FYB/FYB^{ES} 7= AS + FYB^{ES}/FYB^{ES} 8= AS + FYA/FYA 9= AS + FYB/FYB 10= AS + FYA/FYB 11= AS + FYA/FYB^{ES} 12= AS + FYB/FYB^{ES} 13= SS + FYB^{ES}/FYB^{ES}	Discreet quantitative	Ratio / Nominal[a]	Band counting for the combined variables Duffy and HbS mutation for each individual
Protection[b]	1= Double complete (to be 7) 2= Double partial (to be 11 or 12) 3= Single complete (to be 1, 8, 9, 10 or 13) 4= Single partial (to be 5 or 6) 5= None (to be 2, 3 or 4)	Discreet quantitative	Ratio / Ordinal[a]	Combinations of categories for variable "Combination HbS and Duffy"

[a]Scale of the variable once categorized

[b]Categories obtained from the variable "Combination HbS and Duffy"

Table 2. Allelic and Genotypic Frequencies for Duffy and HbS Genes

		Duffy Genotype						
		FYB^{ES}/FYB^{ES}	**FYA/FYA**	**FYB/FYB**	**FYA/FYB**	**FYA/FYB^{ES}**	**FYB/FYB^{ES}**	**Total**
HbS Genotype	**AA**	417[a] 50.9%[b] (49.8-52.1)[c]	24 3,0% (2.6 - 3.4)	18 2.3% (1.9 - 2.6)	50 6.2% (5.6 - 6.8)	148 18.2% (17.2 - 19)	112 13.8% (12.9 -14.6)	769 93.8% (93.2 -94.3)
	AS	32 4.0% (3.5 - 4.45)	0 0.0% (0.0 -0.17)	2 0.36% (0.21- 4.8)	1 0.24% (0.12 - 0.33)	4 6.0% (4.1 - 7.5)	10 1.34% (1.1 - 1.6)	49 6.1% (5.5 - 6.6)
	SS	1 0.24% (0.12 - 0.32)	0 0.1% (0.0 -0.17)	0 0.1% (0.0 -0.17)	0 0.1% (0.0 - 0.17)	0 0.1% (0.0 - 0.17)	0 0.1% (0.0 - 0.17)	1 0.2% (0.1 - 0.3)
	Total	450 55% (54.95 - 56.2)	24 3.0% (2.6 - 3.4)	20 2.5% (2.17 -2.9)	51 6.3% (5.7 - 6.9)	152 18.6% (17.7 - 19.5)	122 14.9% (14 - 15)	**819**
		Duffy allelic frequencies	**FYA=** 15.2% **FYB=** 12.6% **FYB^{ES}=** 72.2%		**HbS allelic frequencies**	**A=** 96.9% **S=** 3.1%		

[a]Absolute frequency; [b]prevalence or relative frequency; [c]credibility region

Table 3. Independence and Regression Tests for the Study Variables

		Variables			
		HbS Mutation	**Duffy Mutation**	**HbS y Duffy combination**	**Protection**
Variables (Fisher's test)	**Sex**	0.8030	0.8731	0.8872	0.8775
	Categorized age	0.0010	0.2603	0.0333	0.0029
	Community	0.0011	0.0000	0.0000	0.0000
Factors (Regression)	**Sex** +	0.6245	0.9345	0.8909	0.7944
	Categorized age +	0.0047	0.1746	0.0846	0.0209
	Community	0.0503	0.0000	0.0001	0.0024

Table 4. "Protection" Variable Prevalence for Each of the 12 Communities of Buenaventura City

		Protection					
		Double complete	**Double partial**	**Single complete**	**Single partial**	**None**	**Total**
Community	**1***	6[a] 18%[b] (13.6 - 21.7)[c]	2 7.7% (4.5 – 10.1)	17 46.1% (40.7 – 51.5)	8 23% (18.3 – 27.9)	4 12.8% (8.9 – 16.0)	37 4.6%[d] (4.12 – 5.1)
	2	1 4.3% (2.12 – 5.8)	0 2.2% (0.63 – 3.0)	25 56.5% (51.5 – 61.5)	16 36.9% (31.8 – 41.6)	2 6.5% (3.8 – 8.4)	44 5.48%[d] (4.9 – 5.9)
	3*	5 10.3% (7.3 – 21.7)	1 3.4% (1.6 – 4.6)	35 62% (57.7 – 66.4)	12 22.4% (18.5 – 25.9)	3 6.8% (4.4 – 8.7)	56 6.94%[d] (6.3- 7.5)
	4	0 1.1% (0.33 – 0.16)	1 2.3% (1.1 – 3.0)	52 60.1% (56.7 – 63.6)	26 30.7% (27.3 – 34.0)	7 9,00% (6.8 – 10.9)	86 10.60%[d] (9.8 – 11.3)
	5	1 3.2% (1.5 – 4.3)	0 1.6% (0.46 – 2.2)	38 62.9% (58.8 – 67.1)	19 32.2% (28.1 – 36.1)	2 4.8% (2.8 – 6.2)	60 7.44%[d] (6.8 – 8.0)
	6*	1 3,00% (1.4 – 3.9)	0 1.5% (0.43 – 2.0)	28 43.4% (39.2 – 47.5)	24 37.4% (33.4 – 36.1)	12 19.4% (15.9 – 22.4)	65 8.0%[d] (7.3 – 8.6)
	7*	3 7.6% (4.9 – 9.7)	1 3.8% (1.8 – 5.2)	20 40.3% (35.7 – 44.7)	13 26.9% (22.6 – 30.9)	13 26.8% (22.5 – 30.8)	50 6.20%[d] (5.6 – 6.7)
	8	2 5.13% (3.0 – 6.7)	3 6.9% (0.43 – 2.0)	25 44.8% (40.2 – 49.1)	24 43.1% (38.7 – 47.4)	2 5.2% (3.0 – 6.8)	56 6.95%[d] (6.3 – 7.5)
	9	2 4,00% (2.3 – 5.3)	1 2.7% (1.3 – 3.7)	46 63.5% (59.9 – 67.3)	16 23% (19.5 – 26.2)	7 10.7% (8.1 – 12.9)	72 8.89%[d] (8.2 – 9.5)
	10	4 6.8% (4.7 – 8.6)	0 1.3% (0.4 – 1.9)	33 46.5% (42.5 – 50.5)	25 35.6% (31.8 – 39.3)	9 13.6% (10.7 – 16.2)	71 8.75%[d] (8.0 – 9.4)
	11	3 6.4% (4.2 – 8.2)	0 1.6% (0.48 – 2.2)	33 54.8% (51.5 – 61.5)	21 35.5% (31.3 – 39.5)	3 6.4% (4.1 – 8.2)	60 7.43%[d] (6.8 – 8.0)
	12*	5 3.6% (2.6 – 4.5)	5 3.6% (2.6 – 4.5)	68 42% (39.5 – 44.6)	56 37.7% (32.3 – 37.2)	28 17.7% (15.5 – 19.6)	162 19.78%[d] (18.9– 20.8)
	Total	33 4.1% (3.6 – 4.6)	14 1.8% (1.5 – 2.1)	420 51.27% (50.0 – 52.4)	260 31.78% (30.7 – 32.8)	92 11.33% (10.5 - 12.0)	**819**

[a]Absolute frequency

[b]Relative frequency or prevalence inside the communities

[c]Credibility region

[d]Calculated frequency related to the total

*Communities that show difference according to the deviance analysis.

Figure 1.

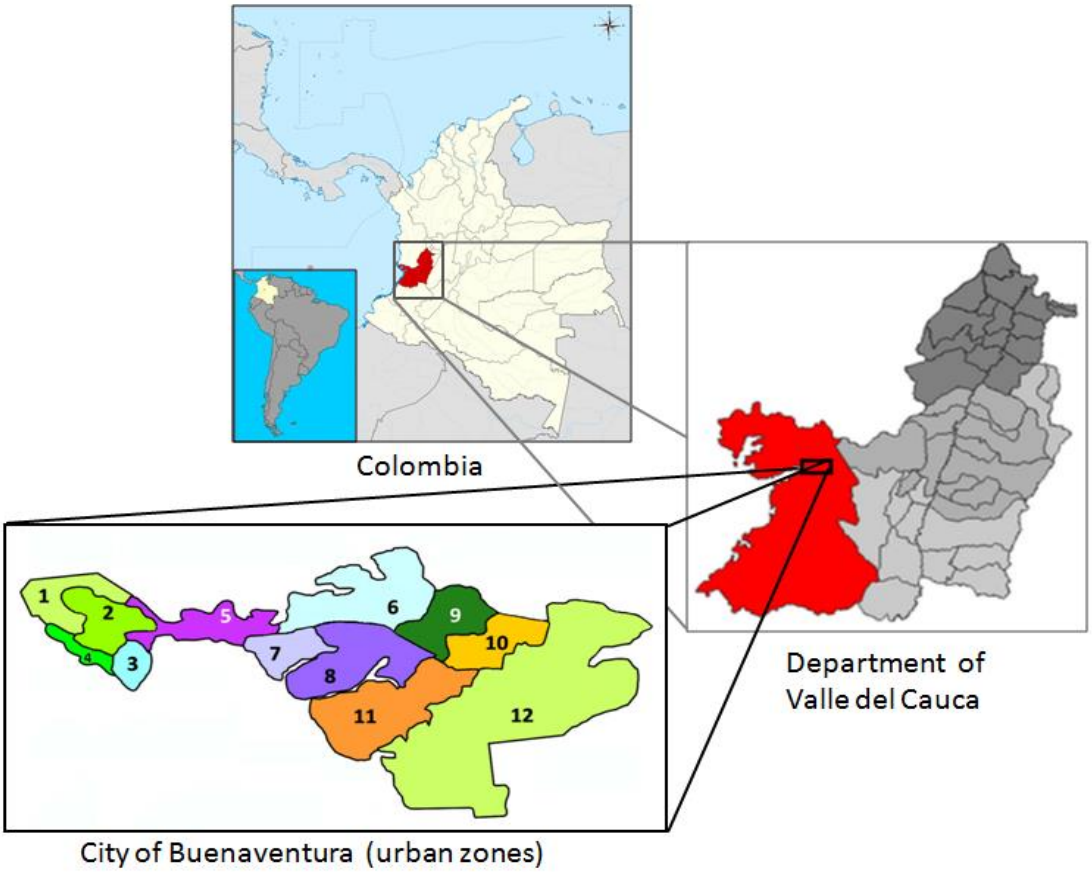

Figure 2.

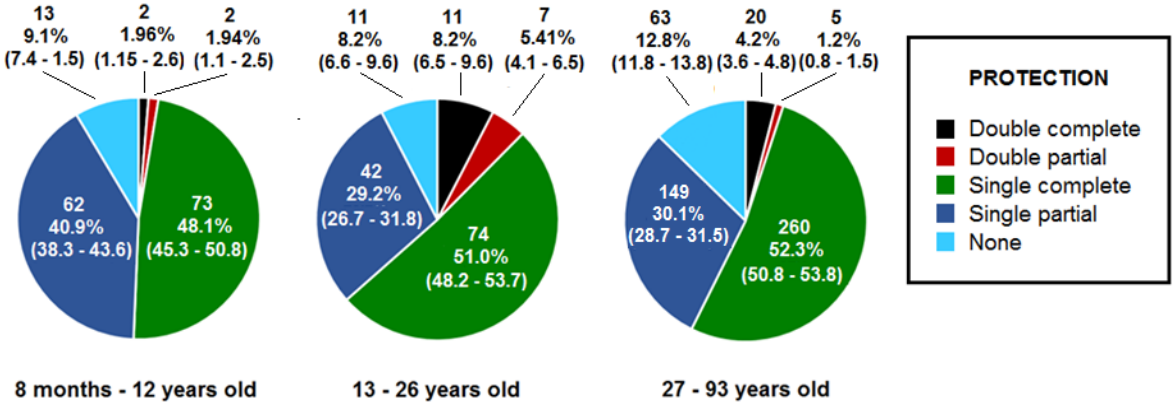

Figure 3.

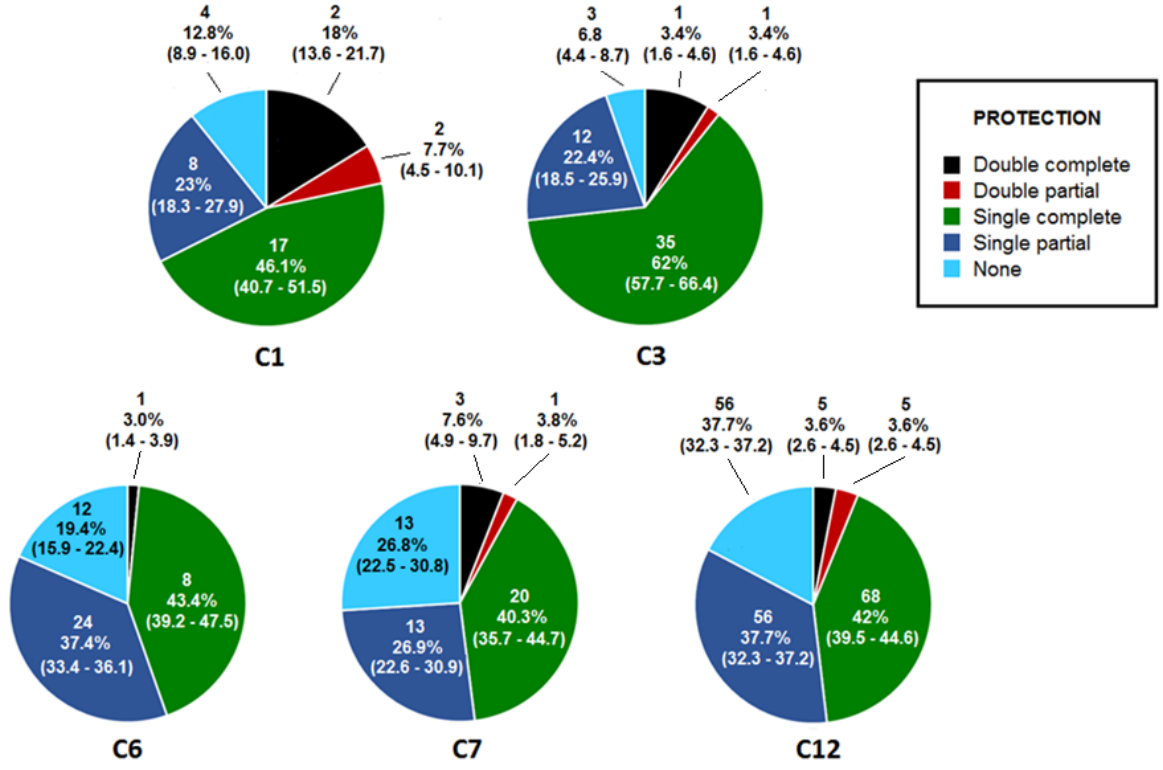

Figure Captions

Figure 1. Map of Buenaventura's city communities, located in the Department of Valle del Cauca (red color). Department located in Colombia (red color), country located in South America. Figure reproduced with permission from Int. J. Genet. 2015.

Figure 2. Pie plot of age groups regarding the 5 levels of protection.

Figure 3. Pie plot of the communities (C) 1, 3, 6, 7, and 12 of Buenaventura regarding the 5 levels of protection.