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Article

Biogeographical ancestry is associated with socioenvironmental conditions and infections in a Latin American urban population

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ABSTRACT

Racial inequalities are observed for different diseases and are mainly caused by differences in socioeconomic status between ethnoracial groups. Genetic factors have also been implicated, and recently, several studies have investigated the association between biogeographical ancestry (BGA) and complex diseases. However, the role of BGA as a proxy for non-genetic health determinants has been little investigated. Similarly, studies comparing the association of BGA and self-reported skin colour with these determinants are scarce. Here, we report the association of BGA and self-reported skin colour with socioenvironmental conditions and infections. We studied 1246 children living in a Brazilian urban poor area. The BGA was estimated using 370,539 genome-wide autosomal markers. Standardised questionnaires were administered to the children's guardians to evaluate socioenvironmental conditions. Infection (or pathogen exposure) was defined by the presence of positive serologic test results for IgG to seven pathogens (*Toxocara spp*, *Toxoplasma gondii, Helicobacter pylori*, and hepatitis A, herpes simplex, herpes zoster and Epstein-Barr viruses) and the presence of intestinal helminth eggs in stool samples (*Ascaris lumbricoides* and *Trichiuris trichiura*). African ancestry was negatively associated with maternal education and household income and positively associated with infections and variables, indicating poorer housing and living conditions. The self-reported skin colour was associated with infections only. In stratified analyses, the proportion of African ancestry was associated with most of the outcomes investigated, particularly among admixed individuals. In conclusion, BGA was associated with socioenvironmental conditions and infections even in a low-income and highly admixed population, capturing differences that self-reported skin colour miss. Importantly, our findings suggest caution in interpreting significant associations between BGA and diseases as indicative of the genetic factors involved.

Introduction

Racial inequalities in health are observed for different outcomes, with race and ethnicity being traditionally considered a fundamental axis for the study of health inequalities in Europe and the United States, and more recently in Brazil (Chiavegatto Filho, Beltran-Sanchez & Kawachi, 2014; Hussey, Anderson & Berthelot, 2008). Although the socioeconomic status (SES) accounts for much of this difference, racial discrimination and putative genetic factors, in some cases, may also be involved (Collins, 2004). In Brazil, whose mixed population results from more than 500 years of physical, social and commercial contact between Europeans, Africans and Native Americans (Salzano, 2004), the classification based on skin colour is closely associated with the concept of race, and race and skin colour are used as interchangeable terms in epidemiological studies (Kabad, Bastos & Santos, 2012). Indeed, since the mid-20th century, the Brazilian Institute of Geography

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and Statistics (IBGE) classifies individuals by self-reported "cor" (a Portuguese word used in Brazil) or race, a concept that involves a mix of perceptions that include ancestry, morphological traits (including skin colour) and cultural issues (Telles, 2002). However, the racial definition is dependent on a myriad of factors, such as self-perception, ascription by others, and institutional and cultural contexts (Brunsma, 2005; Jenkins, 1994). Moreover, the way individuals define themselves about their race/skin colour may not match the classification made by a third party (Telles & Lim, 1998).

Despite the issues involved with self-reported skin colour, in Brazil, as in other countries, it has traditionally been used in epidemiological studies as a proxy for biogeographical ancestry (BGA) (Pena, Di Pietro & Fuchshuber-Moraes, 2011), which is defined as proportions of the individual genome inherited from each of the continental groups that contribute to the formation of the admixed population (Royal, Novembre & Fullerton, 2010). In turn, the advent of high-throughput genotyping technologies and cataloguing of human genetic diversity in public databases have enabled the selection of ancestry informative markers (AIMs), which are used to estimate the geographical origins of one's ancestors and the proportion of individual genome inherited from each these ancestors (Shriver, Parra & Dios, 2003). This approach provides a more objective estimate of the ancestral origins of individuals than self-reported skin colour. Furthermore, because BGA is related to self-reported skin colour, although the magnitude of this correlation varies among different populations (Lima-Costa, Rodrigues & Barreto, 2015; Parra, Kittles & Shriver, 2004), it can be used as a surrogate for genetic factors underlying the ethnoracial differences to disease risk. Indeed, in recent years, there has been increasing interest in association studies between individual ancestry inferred by genetic markers and complex diseases (Via, Ziv & Burchard, 2009). However, given the multifactorial nature of many of these outcomes, knowledge about the relationship between BGA and social determinants of health is essential to avoid being attributed to genetic variants differences to the risk of diseases that are due to non-genetic determinants that covariate with BGA (Risch, Burchard & Ziv, 2002). However, studies of the relationship between the individual BGA and SES, indicator variables of housing and living conditions and infections are scarce. Similarly, the comparison between BGA and self-reported skin colour as proxies for these factors has also been poorly investigated. The Social Changes, Asthma and Allergies in Latin America (SCAALA) cohort has a range of information on social and environmental factors that play an essential role in the development of asthma and allergies among children and adolescents (Barreto, Cunha & Alcantara-Neves, 2006). This information offers an opportunity to investigate and compare the association of BGA and self-reported skin colour with different variables. Thus, the present study aimed 1) to study the association of BGA with SES, environmental conditions and infection by pathogens; and 2) to compare the association between BGA and self-reported skin colour with these different factors.

Methods

Study population and data collection

This study was performed in the city of Salvador in Northeastern Brazil, which has 2.8 million inhabitants, of which more than 80% are self-declared admixed or black according to the last official census conducted in 2010 (SIDRA, 2016). The design of this study has been reported elsewhere (Barreto et al., 2006). Briefly, the study population consists of 1246 children previously studied to evaluate the effect of a sanitation programme and enrolled when they were 0–3 years old from 1996 to 2003 (Barreto, Genser & Strina, 2007). Standardised questionnaires were administered to the children's guardians between 1997 and 2003 (baseline), and data were collected on housing, sanitation, and socioeconomic conditions (Strina, Cairncross & Barreto, 2003). Data collection was repeated in 2005 when the children were aged 4–11 years, at which time blood samples were collected, and serological and parasitological tests were performed. Concerning tap water and household sewage systems, we obtained data from two different time points: early life (i.e., < 3 years old) and later childhood (age range, 4–11 years).

Infection (or pathogen exposure) was defined by the presence of positive serologic test results for IgG to seven pathogens (*Toxocara spp*, *Toxoplasma gondii, Helicobacter pylori*, and hepatitis A, herpes simplex, herpes zoster and Epstein-Barr viruses) and the presence of intestinal helminth eggs in stool samples (*Ascaris lumbricoides* and *Trichiuris trichiura*). We used a threshold of 3 or fewer infections to distinguish light from heavy infection, as reported elsewhere (Janson, Asbjornsdottir & Birgisdottir, 2007).

To compare the observed associations of self-reported skin colour and BGA with the different outcomes evaluated in childhood, as well as to evaluate the concordance between BGA and self-reported skin colour, a subsample of 878 individuals with BGA information and aged between 12 and 19 years during the most recent SCAALA-Salvador survey (year 2013) was used. In this case, African BGA was categorised using tertiles. These 878 individuals answered the question "What is their race/colour?" with five response options (white, admixed, black, yellow, and indigenous or "branco", "pardo", "preto", "amarelo" and "indígena" in Portuguese, respectively) and 69 self-reported white, 377 self-reported admixed, 432 self-reported blacks and no respondences self-reported yellow or indigenous. No statistically significant differences were observed between individuals with and without skin colour information regarding the proportions of ancestry as well as the different outcomes investigated (Supplementary Table 1). Ethical approval for this study was obtained from the Brazilian National Ethical Council, and written informed consent was obtained from the guardian of each child. Moreover, research has been conducted according to the principles outlined in the Declaration of Helsinki.

Genotyping and BGA estimation

DNA was extracted from peripheral blood using a commercial kit (Gentra® Puregene® Blood Kit (Qiagen)), and samples were successfully genotyped using Illumina platforms (San Diego, California) with the Omni 2.5 M array. To estimate the contribution from Africans, Europeans and Native Americans to each individual in the study population, we used the ADMIXTURE software (Alexander, Novembre & Lange, 2009). We performed unsupervised tri-hybrid (k = 3) ADMIXTURE analyses based on 370,539 SNPs shared by samples from the HapMap Project, Human Genome Diversity Project (HGDP) (Li, Absher & Tang, 2008; Altshuler, Gibbs & Peltonen, 2010) and the study population. We did not perform the tests for association with Amerindian ancestry, as the proportion of Amerindian ancestry in our population was too low (median 5.92%, interquartile range 4.26% - 7.91%) for such tests to be meaningful.

Statistical analyses

Due to the measurement scale of BGA, all initial analyses were performed using a non-parametric approach, such as the Pearson chi-square test and Mann–Whitney test. Medians were used as a descriptive measure to characterise the distribution of BGA as a function of the different outcomes investigated. To estimate the magnitude of the association between the proportion of African/European ancestry and the different outcomes, bivariate analyses were performed using binary or multinomial logistic regression (for the politomic variables Mother Education and Income). Odds Ratio (OR) measures and their 95% confidence intervals were calculated for each 20% increase in individual BGA, using the formula $e^{(\beta * 20)}$, where e is the base of the natural logarithms and β is the coefficient of binary or multinomial logistic regression. The concordance between the self-declared skin colour and African ancestry tertiles was estimated using the kappa

Table 1
Odds Ratios for socio-environmental conditions and infections according to the proportion of individual African and European ancestry.

Biogeographical ancestry	Outcomes
	OR (95% CI)
Sociodemographic factors	
	Mother education (base outcome: Elementary education)
	More than elementary education
African Ancestry	**0.78 (0.64–0.97)**
European Ancestry	**1.27 (1.01–1.59)**
	More than high school
African Ancestry	**0.58 (0.46–0.73)**
European Ancestry	**1.84 (1.44–2.36)**
	Income (base outcome: < 1 minimum wage)
	1–2 minimum wage
African Ancestry	**0.74 (0.61–0.89)**
European Ancestry	**1.38 (1.13–1.68)**
	> 2 minimum wage
African Ancestry	**0.59 (0.47–0.74)**
European Ancestry	**1.85 (1.44–2.36)**
Enviromental factors	
	Tap water never or at most in one time point
African Ancestry	**1.52 (1.24–1.86)**
European Ancestry	**0.62 (0.49–0.78)**
	Sewage system never or at most in one time point
African Ancestry	**0.84 (0.72–0.99)**
European Ancestry	1.18 (1.00–1.40)
	Presence of rodents in home
African Ancestry	**1.45 (1.23–1.71)**
European Ancestry	**0.65 (0.54–0.78)**
Infections	
	Heavy infection burden
African Ancestry	**1.65 (1.34–1.92)**
European Ancestry	**0.59 (0.49–0.72)**
	Presence of helminth infection
African Ancestry	**1.43 (1.18–1.71)**
European Ancestry	**0.67 (0.54–0.81)**

coefficient. A level of significance of 5% ($\alpha = 0.05$) was adopted. The analyses were performed using Stata software, version 12.0 (StataCorp, College Station, TX, USA).

Results

The majority of the study population consisted of boys (52.6%). The median distribution of BGA in the overall study population was African 50.74%, European 42.34% and Amerindian 5.92%. The general characteristics of the study population and median proportion of BGA according to socioeconomic status, environmental variables and infections are presented in Supplementary Table 2.

The OR of different outcomes investigated due to a continuum of BGA is shown in Table 1. Each 20% increase in African ancestry was negatively associated with higher maternal education (OR for *"More than elementary education"* = 0.78; 95% CI 0.64 - 0.97, and OR for "*More than high school*" = 0.58; 95% CI 0.46 - 0.73) and household income (OR for *"1–2 minimum wage"* = 0.74; 95% CI 0.61–0.89, and OR for *" > 2 minimum wage"* = 0.59; 95% CI 0.47–0.74). Regarding environmental variables, each increment of 20% in individual African ancestry increased the chance of never having tap water in the household or, at most, having it at one time-point (OR = 1.52; 95% CI 1.24–1.86) as well the chance of a reported presence of rodents in the home (OR = 1.45; 95% CI 1.23–1.71). A negative association, in turn, was observed between African ancestry and the absence of a sewage system at home (OR for *"never have sewage system or at most have in one-time point"* = 0.84; 95% CI 0.72–0.99). A greater chance of heavy infection burden (OR = 1.65; 95% CI 1.34–1.92) and helminth infections (OR = 1.43; 95% CI 1.18–1.71) was also observed with an increase in African ancestry proportion in children. European ancestry was strongly and negatively correlated with African ancestry (rho = −0.97), and thus opposite results were observed for the association of European ancestry with the outcomes investigated (Table 1). An exception was the outcome sewer system at home, for which no association was found with European ancestry. The association between BGA and these different outcomes remained relatively unchanged in logistic models adjusted for sex and age (data not shown).

Table 2
Association of self-reported skin color and African ancestry tertiles with socio-environmental conditions and infections.

Skin color	Outcomes	African Ancestry	Outcomes
	OR (95% CI)		OR (95% CI)
Sociodemographic factors			
	More than elementary education		More than elementary education
Admixed	0.63 (0.30–1.31)	2nd tertile	**0.56 (0.36–0.87)**
Black	0.69 (0.33–1.44)	3rd tertile	**0.50 (0.32–0.79)**
	More than high school		More than high school
Admixed	0.55 (0.26–1.18)	2nd tertile	**0.44 (0.28–0.71)**
Black	0.56 (0.26–1.19)	3rd tertile	**0.40 (0.25–0.65)**
	1–2 minimum wage		1–2 minimum wage
Admixed	1.01 (0.56–1.80)	2nd tertile	**0.67 (0.46–0.97)**
Black	0.81 (0.45–1.44)	3rd tertile	0.77 (0.53–1.12)
	> 2 minimum wage		> 2 minimum wage
Admixed	1.04 (0.51–2.14)	2nd tertile	**0.54 (0.35–0.85)**
Black	0.89 (0.44–1.81)	3rd tertile	**0.61 (0.39–0.96)**
Enviromental factors			
	Tap water never or at most in one time point		Tap water never or at most in one time point
Admixed	1.39 (0.68–2.85)	2nd tertile	1.00 (0.66–1.54)
Black	1.53 (0.75–3.11)	3rd tertile	1.34 (0.89–2.01)
	Sewage system never or at most in one time point		Sewage system never or at most in one time point
Admixed	0.86 (0.51–1.47)	2nd tertile	0.98 (0.70–1.36)
Black	0.77 (0.45–1.29)	3rd tertile	0.79 (0.57–1.10)
	Presence of rodents in home		Presence of rodents in home
Admixed	**1.81 (1.08–3.05)**	2nd tertile	**1.94 (1.39–2.71)**
Black	1.56 (0.93–2.62)	3rd tertile	**1.86 (1.33–2.58)**
Infections			
	Heavy infection burden		Heavy infection burden
Admixed	**2.71 (1.30–5.65)**	2nd tertile	1.33 (0.91–1.93)
Black	**3.54 (1.71–7.34)**	3rd tertile	**2.30 (1.60–3.30)**
	Presence of helminth infection		Presence of helminth infection
Admixed	1.90 (0.93–3.87)	2nd tertile	1.20 (0.82–1.76)
Black	**2.59 (1.29–5.23)**	3rd tertile	**1.60 (1.11–2.32)**

References: Skin color (Whites); African Ancestry (1st Tertile)

The results of the association between self-reported skin colour and BGA with the different outcomes are shown in Table 2. Self-reported skin colour and African ancestry were significantly associated with infection burden and helminth infections. Maternal education and income were significantly associated with African ancestry but not with the self-reported skin colour. African ancestry was also associated with the presence of rodents in the household, and a significant association was observed comparing admixed versus whites, but not blacks versus whites for this outcome. None of the other variables was significantly associated neither with self-reported skin colour nor with African ancestry. Although significant, concordance between the African ancestry tertiles and self-reported skin colour was low (kappa = 0.171; $p < 0.001$) (Table 3).

The results of the association between African ancestry (continuous) and the different outcomes according to the skin colour categories are shown in Fig. 1. Among the self-declared blacks, African ancestry was positively associated with the presence of rodents in the home (OR = 1.48; 95% CI 1.07 - 2.05) only. Among the self-declared admixed, in turn, every 20% increase in the proportion of African ancestry significantly increased the chances of the presence of rodents at home (OR = 1.99; 95% CI 1.40–2.82), heavy infection burden (OR = 1.56; 95% CI 1.09–2.25) and helminth infections (OR = 1.50; 95% CI 1.03–2.20).

Table 3
Agreement between the self-reported skin color and African ancestry tertiles.

Self reported skin color, n (%)	African ancestry			Pvalue	Kappa
	1st tertile	2nd tertile	3rd tertile		
White	49 (71.0)	15 (21.7)	5 (7.2)	< **0.0001**	0.171
Admixed	181 (48.0)	125 (33.2)	71 (18.8)		
Black	56 (13.0)	155 (35.9)	221 (51.2)		

In addition, African ancestry was negatively associated with higher levels of income (OR for *" > 2 minimum wage"* = 0.55; 95% CI 0.34–0.87) and higher maternal education (OR for *"More than high school"* = 0:49; 95% CI 0.31–0.78 and OR for *"More than elementary education"* = 0.58; 95% CI 0.38–0.89).

Discussion

Our study has shown that individual African ancestry was negatively associated with SES, as measured by maternal education and household income and the inverse occurred when European ancestry was considered. The lower socioeconomic status of those with an increasing proportion of individual ancestry derived from groups that were dominated and enslaved by Europeans during the colonial period (Amerindians and Africans) was previously reported to other admixed populations (Campbell, Parra & Duque, 2012; Florez, Price & Campbell, 2009; Sanchez, Rasmussen & Riba, 2012; Via, Gignoux & Roth, 2011). In our study, African ancestry was also associated with poor environmental conditions (not having had tap water and having rodent infestation in the home) and with a high burden of infections. These findings demonstrated the persistence of a social stratification related to BGA. Individuals with higher African ancestry occupied more disadvantageous positions and suffered from prejudice compared to those with less African ancestry. Interesting, the population of the city of Salvador, where the current study was performed, is highly admixed, with everyone having some level of admixture (Lima-Costa et al., 2015).

Although self-reported skin colour was associated with income and educational attainment in studies that analysed samples from national surveys in Brazil (Arias, Yamada & Tejerina, 2004; Bailey, Loveman & Muniz, 2013; Marteleto, 2012), in the present study, no association was observed between SES and self-reported skin colour. Furthermore, self-reported skin colour was significantly associated with infections but not with variables indicating exposure to dirt and poor environmental conditions. Otherwise, even when African ancestry was categorised based on tertiles of a continuous distribution, significant associations were observed for most of the outcomes investigated in the subsample of individuals with data for self-reported skin colour. In addition, when BGA was used as a continuous variable in this same universe of 878 individuals, the same pattern of association with outcomes was observed as in the global sample (Supplementary Table 3). These results can be explained by some hypotheses discussed below.

First, because the income gap between whites and non-whites in Brazil is more prominent at the upper ends of the wage distribution (Bailey et al., 2013), the observed lack of association between skin colour and SES may be because most of our study population has low-income and therefore is in the bottom of the distribution. Second, skin colour self-identification can be a less appropriate proxy for on how others classify one's race (Telles & Lim, 1998). Because racial discrimination and damages resulting from it are more dependent on the way in which others classify people regarding their race/colour (Telles & Steele, 2012), self-reported skin colour could attenuate the differences between whites and non-whites for the outcomes investigated here. Indeed, different studies have shown that racial inequities to income, for instance, are higher when the skin colour rating is performed by an external observer (hetero-classification) compared to the differences in income based on self-reported skin colour (Telles & Lim, 1998; Bailey et al., 2013).

In sequence, the important question raised by our findings is why BGA but not self-reported skin colour was associated with most outcomes investigated. An additional issue of the official system of racial classification used in Brazil is that it ignores potential phenotypic intragroup heterogeneity (Telles & Steele, 2012). Once the skin colour phenotype encompasses a much more diverse continuum of variation than that captured by racial categories, individuals with different complexions can be classified in the same category but have different

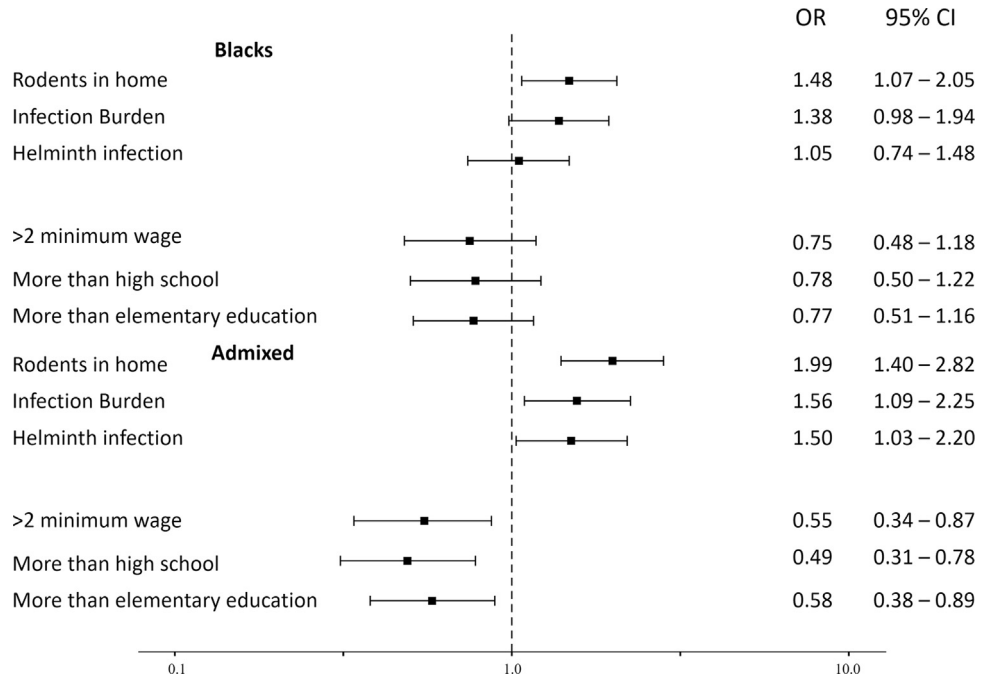

Fig. 1. Association of African biogeographical ancestry with the different outcomes investigated according to self-reported skin color. OR: Odds Ratio for each 20% increase in individual African ancestry.

life chances due to their skin tone. Indeed, different studies performed in African-American and Latino populations in the US and based on interviewer-rated skin colour have shown that lighter-skinned individuals enjoyed higher income and educational attainment compared to their darker-skinned counterparts (Gómez, 2004; Keith & Herring, 1991; Murguia & Telles, 1996; Espino & Franz, 2002). Reported experiences of discrimination by whites were also more common among Latinos who were dark-skinned than among those of lighter skin in the US (Arce, Murguia & Telles, 1987). A recent and, to the best of our knowledge, first study of its kind performed in Latin America reported that darker skin colour was negatively and consistently associated with educational attainment in all evaluated countries, including Brazil (Telles, Flores & Urrea-Giraldo, 2015). Taken together, these findings suggest that individual BGA could be a proxy for the intensity of skin pigmentation and other phenotypic characteristics essential for determining race, particularly for the ascription of one's race by others (Guimarães, 2012). Furthermore, BGA could also act as a proxy for the origin of social class, since race and social class work together to shape socioeconomic stratification in the Americas. Importantly, social class is also the result of historical processes, such as slavery and other systems of domination to which blacks and Native American groups were subjected (Telles et al., 2015). Thus, in our population, compared to self-reported skin colour, BGA could capture both the effects of racial accumulated privileges and disadvantages acquired in past and contemporary structural and ideological factors that perpetuate those historical injustices, such as current discriminatory processes in labour and educational markets. These results are consistent with the fact that in Brazil, self-reported race/colour groups do not adhere to any rigid descent rule, unlike the hypodescent or "one drop" rule based on the biological ancestry of individuals as already used in the US (Guimarães, 2012). Indeed, a low concordance was observed when analysing the association between self-reported skin colour and the tertiles of African ancestry. However, importantly, self-reported skin colour was significantly associated with both helminth infections and infection burden. Furthermore, a greater risk of the presence of rodents at home among self-reported admixed compared to whites was also observed. This finding underscores the importance of using race/skin colour as a marker of exposure to factors that affect the health status in epidemiological studies, particularly since self-reported race/skin colour takes into account subjective and contextual factors that would otherwise be difficult to capture through BGA.

The hypothesis developed above could also help to explain the associations between BGA and different outcomes when stratifying by self-reported skin colour. In this sense, individuals with a higher proportion of African ancestry would be more likely to have a less privileged social origin and present specific phenotypes, such as darker skin colour and thus have greater chances of exposure to poverty, precarious living conditions, and infections. According to our data, it seems to be particularly important among the people who self-declared as admixed, which appears logical when one considers that this is a group intermediary between whites and blacks representing a diverse range of products of admixture (Osório, 2008). However, although not statistically significant, these same trends were observed among self-reported blacks, requiring further investigation in more empowered studies.

The present study has some limitations. First, the sample consisted of adolescents and young adults 12 to 19 years of age who are less likely to have experienced racial discrimination in the labour and educational markets. This may have led some black individuals to self-declared as admixed or white, which could neutralise potential differences between these groups for the analysed outcomes. Second, the relatively small sample size impaired an evaluation of the investigated associations among those individuals self-reported as whites. Third, information on skin colour classification made by a third person (hetero-classification) or on skin pigmentation as measured by reflectometry were not available. This precluded some of the hypotheses discussed here from being tested for our population. Finally, caution should be taken to the generalisation of the results reported here to other populations with distinct historical and sociodemographic backgrounds.

In conclusion, we observed that in an urban and low-income population of Latin America, an increased proportion of individual African ancestry was significantly associated with poverty, poor environmental conditions and infections by pathogens. An inverse association was observed when the proportion of individual European ancestry was considered. Finally, our results indicate the need for caution when interpreting results from associations between BGA and any diseases. As shown, BGA is strongly associated with factors belonging to the most different environmental and social dimensions that can potentially affect individual health status. Failure to observe and take into account these factors can lead to confounders in these studies, attributing to genetic factor differences that result from socio-historical processes, which covariates with BGA.

Acknowledgments and contributors

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Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The project was approved by the ethics committees at the Federal University of Bahia (register 003–05/CEP-ISC) and National Council for Ethics in Research (CONEP, resolution number 15 895/2011). Moreover, the research has been conducted according to the principles expressed in the Declaration of Helsinki.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ssmph.2018.03.006.

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