Genetic Ancestry of the Panamanian Population: Polymorphic Structure, Chibchan Amerindian Genes; and Biological Perspectives on Diseases

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Abstract

Panama's unique geographic position has subjected the country's population to a complex admixture process since pre-Columbian times. Ethno-historical records indicate that migratory waves from Spain and Western African ancestral groups are part of the Panamanian gene pool. Previous reports suggest that several Amerindian groups participated in the miscegenation process but their specific contribution to the genetic background is not well understood because multiple tribes and languages occupied Panama. Moreover, historical records are unclear and most Amerindian tribes suffered extinction. It is thought that by the arrival of Europeans, Chibchan and Cueva were the largest Amerindian groups inhabiting Panama. Chibchan occupied the Western region of Panama and their modern descendants are the Ngöbe Amerindians. Cueva Amerindians inhabited the Eastern Panama however, they are extinct but they are ethno-linguistically associated to Chibchan or with Chocoan-speaking Amerindian groups. In order to identify Amerindian genes in the mestizo population, we gathered genetic data from Ngöbe and Embera Amerindians, belonging to Chibchan and Chocoan Amerindian families, respectively. Fifteen polymorphic STR autosomal markers were analyzed to characterize in detail the genetic structure and admixture of the country's population. We found that the population of Panama shows relatively high levels of contribution from all three ancestral populations: 24% African, 25% European and 51% Amerindian. The Amerindian genetic component is from Chibchan origins showing high distribution within Western and Eastern regions. We propose that Chibchan genes were mostly passed to modern mestizos from Ngöbe ancestors and extinct Cueva Amerindian groups. Overall, Panamanian mestizos are highly polymorphic and differentially admixed among the country's provinces as evidenced by diversity parameters and genetic distances calculated. Given these highly diverse admixture patterns among provinces, we surmised that they might be also associated with differences in disease incidence. Therefore, we calculated parameters of biomedical relevance including forensic and epidemiological data of the major diseases in the population and focused on prostate cancer and cerebrum-cardiovascular disorders. Prostate cancer showed higher incidence in provinces with the highest African admixture and cerebrum-cardiovascular disorders showed higher incidence in provinces with the highest European admixture and moderately high African genes. Overall, these results suggest that genetic ancestry proportions shaped by ethno-historical events might play a significant impact on the health/cultural lifestyles and disease patterns of these populations.

INTRODUCTION

Panama's unique geographic position has subjected the country's population to different evolutionary processes since Pre-Columbian times. The population of Panama has suffered one of the most intricate processes of races and culture amalgamation in the Americas within a relatively small population and territory. However, the admixture component of current population is not well understood. The first attempt to determine the gene admixture of the Panamanian population was by Arias et al. (2002) with a sample of 4,200 subjects from across the country but using only two genetic systems of classical markers (A-B-0 and Rh). In addition, these findings might not be representative of the current population of Panama since the population of Panama in the early 2000s was estimated in about 3.05 million, whereas the most recent estimates of 2016 indicate a boom being now estimated in 4.04 million, according to the Panama's National Institute of Statistics and Census (NISC). In more recent years, a few genetic studies focused on mitochondrial DNA (mtDNA) (Perego et al. 2012) and Y-

chromosome (Grugni etal. 2015) analysis of modern Panamanians. Those studies showed that the majority of mtDNA lineages (83%) are Amerindians with only 14% African and 2% European (Perego et al. 2012). Furthermore, Y-chromosome studies showed 50% contribution of Amerindian, and about 44.1% West-Eurasian with other minor or non-predictable groups (Grugni etal. 2015). However, although these findings are interesting in terms of the information generated, they still do not make clear the admixture problem since the use of uniparental markers might not be representative of the autosomal gene pool of modern Panamanians. For instance, Arias et al. (2002) showed that the admixture of Panama in early 2000s was 38% of African, 36% Amerindian and 25% European genes, thus suggesting that the admixture patterns of modern Panamanians might be more complex.

It is known that during European conquest and soon after the discovery of the Pacific Ocean by Vasco Núñez de Balboa in 1513, Panama became a strategic marketplace and port of exchange of silver, gold, slaves and merchandises transported from South America (Panama city, on the Pacific shore) through the Camino de Cruces (Road of the Crosses) across the isthmus to Portobelo on the Caribbean shore and then shipped to Europe and vice versa (Poveda-Ramos, et al., 2004). This intense commerce activity promoted other events such as massive migratory wave patterns, together with cultural and racial admixture. In the beginning, admixed people (mestizos) were a minority, but ultimately mestizos dominated the majority of the population until present days. Several minorities participated in admixture of Panama, but the three major ancestral groups of this admixed population are ethno-historically grouped as Europeans, Africans and Amerindians (Jaén-Suárez, 1978; 1998).

The European genetic contribution came mainly from many thousands of Spaniards (mainly men, thus highly contributing to the Y-chromosome gene input) that arrived during and following the conquest. The first Africans arrived to Panama from Western Africa to replace the Amerindian male slaves, which helped the survival and recovery of Amerindian males after the bottleneck and further contributed to the Y-chromosome genes. Several approximations estimate that between the 16th and 17th centuries more than 35,000 Africans arrived to Panama, however, since contraband was very common, it is thought that the real numbers are much higher (Jaén-Suárez, 1978). The second wave of Africans (African-Caribbean) came to Panama during the California gold rush between 1850 and 1855 for the construction of the Panama Railroad. More than 80,000 African-Caribbean workers arrived from the Caribbean islands but most of them returned to their countries. The third African (African-Caribbean) migration wave was associated with the construction of the Panama Canal between 1881-1914 (Maloney, 1993). More than 60,000 workers were employed, of which 44.1% were from Barbados, 24% were from all other Antilles, 25% from Europe, of which 18% were Spaniards, and the remaining 6.9% were from Central and Latin America (Maloney, 1993). These numbers were very significant in those times, since the entire population of the country was estimated at 316,054 inhabitants (Maloney, 1993).

The Amerindian genetic ancestors of modern mestizos of Panama are remounted before the conquest of Panama in 1510 by Rodrigo de Bastidas. When Spaniards arrived to the Isthmus of Panama, there were multiple small Amerindian groups populating the area. Ethno-historical and demographic data indicate that nearly 500,000 Amerindians populated Panamanian lands in the beginning of 16th century but Chibchan and Cueva-language groups were the two largest Amerindian populations inhabiting the isthmus (Jaén-Suárez, 1998). Archeological and ethno-historical reports indicate that by the time of the Spaniard conquest, many Chibchan groups inhabited the South-Western portion of the isthmus from the Western portion of the Coclé Province to the Eastern part of Northern Costa Rica. Based on ethno-historical and linguistic data it is known that Chibchan's descendents are the still-living Ngöbe Amerindians (also known as Ngawbe-Guaymi), among other smaller tribes (Jopling, 1994; Cooke, 1982, Barrantes et al., 1990; see maps on Figure 1). Ngöbe population is a well-characterized Amerindian group belonging to the Chibchan-speaking linguistic branch and still inhabits Western Panama (Approximately 260,000 inhabitants, Panama's NISC) and small regions of Costa Rica (Barrantes et al., 1990, Jorge et al., 2002). The Eastern side of the isthmus was inhabited by other large Amerindian group, the Cueva-language group (or simply Cueva), from the Gulf of Urabá and the Atrato River (Colombia) to the "Indio River" on the Caribbean side of Panama and the Mata Ahogado River on the Pacific side of Panama (Romoli, 1987, Jopling, 1994), (Figure 1). It is thought that unfortunately, Cueva people were extinct as a tribe by 1550, mainly due to diseases, slavery and wars against Spaniards (Romoli, 1987). Their language group is not clear but Cueva are often referred as Chocoan/Paezan-

Speaking but others think they were Chibchans (Loewen, 1963, Constenla, 1991). The role of these two major Amerindian families is not well-understood and it has not been accurately addressed in previous admixture studies, thus it remains the question of how different ancestral Amerindian populations contributed to the current gene pool.

To determine the ancestry of modern Panamanian mestizos we analyzed 15 polymorphic short tandem repeats (STR) autosomal markers and characterize in detail the population genetic structure and admixture per province/regions and the total country. Additionally, we address the question of the Amerindian tribes (Chibchan or Chocoan) ancestry of the modern mestizo population. We determined that the Panamanian population is composed of 24% African, 25% European and 51% Amerindian genes. The entire Amerindian component is from Chibchan origins. Panamanians are highly polymorphic and diverse among the country's provinces. We thought that these differences in admixture among provinces might be also associated with differences in disease proportions. Therefore, we calculated parameters of biomedical significance including forensic and epidemiological data of the major diseases affecting the population. We focused on prostate cancer and cerebrum-cardiovascular disorders and found that prostate cancer showed higher incidence in provinces with the highest African admixture; whereas cerebrum-cardiovascular disorders showed higher incidence in provinces with the highest European admixture and moderately high African genes. Altogether, these findings suggest that genetic ancestry variability, shaped by ethno-historical events, might play an important role on the health disparities and cultural lifestyles leading to the disease patterns differences found on these populations.

MATERIALS AND METHODS

Selection of Mestizos Volunteers

In Panama, most mestizo population is concentrated in the major urban zones. We therefore collected blood samples from about 800 unrelated mestizos (admixed) subjects from the main cities of seven provinces in the Republic of Panama: Panama, Colón, Coclé, Herrera, Los Santos, Veraguas and Chiriquí. Individual subjects, sample collections and management were processed in accomplishment of the international standards suggested by the DNA Commission of the International Society for Forensic Genetics (DNA Commission 1994; Bar et al. 1997). Individuals selected for the study were interviewed to confidentially record biographic information, which included names, birthplaces and genetic ancestry of their parents and grandparents. Individuals that were not born in Panama or whose both parents were not Panamanian were not considered for the study. Additionally, individuals belonging to any Amerindian tribe or with an obvious recent origin from other regions such as Asia were not included for the analyses. Appropriate informed consent was obtained from the selected individuals. Due to inaccessibility, two provinces could not be included in this study: Bocas del Toro and Darien. However, these provinces are relatively small in population number, representing only 3.2% (Bocas del Toro) and 1.2% (Darien) of the population in the entire Republic of Panama, according to NISC. Additionally, NISC's data indicates, that Bocas del Toro and Darien are mainly composed of Amerindians and Africans descendants, who are relatively isolated, thus showing low levels of admixture with most mestizo population. Therefore, the lack of data from Bocas del Toro and Darien provinces does not affect significantly the overall results for the country, because of success in sampling the provinces were inhabits more than 95% of the mestizo population.

Blood Collection, PCR Amplification and Genotyping

Peripheral blood was collected and stored in tubes containing EDTA anticoagulant. Then, using plastic pipettes, blood was placed onto the center of each of four circles of commercially prepared FTA Cards (DNA Testing Center Inc. Euless, Texas). FTA paper was cut in ~2-mm-diameter and washed according to the procedure specified by the manufacturer. PCR amplifications were performed using directly the washed small piece of FTA card. Polymerase chain reactions were performed on an Applied Biosystems 9700 model Thermal Cycler. All PCR amplifications were carried out in a 25-uL total volume containing a ~2-mm-diameter piece of FTA paper. Fifteen STR autosomal polymorphic loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, vWA, TPOX, D18S51, D5S818, FGA, D19S433 and D2S1338 were genotyped by multiplex PCR reactions, using a commercial AmpFISTR Identifiler kit (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's protocols. Amplified products and reference ladders provided by the kit were analyzed by capillary electrophoresis using the ABI PrismTM 310 Genetic Analyzer.

Gene Admixture Model and Ancestral Population Data

Several world populations have participated in the admixture processes of Panama. However, extensive evidence supports that the major ancestral components of this admixed population are Europeans from Spain, Western-Africans, and Amerindians. Previous population studies using ABO and Rh blood systems (Arias et al., 2002), mtDNA (Perego et al. 2012) and Y-chromosome (Grugni etal. 2015) also supports this trihybrid ancestral pattern. We have therefore designed a trihybrid model of ancestry for our analyses. As mentioned above, ethno-historical and genetic data indicate that Spaniards brought the European genetic component to Panama. To test the Spanish genetic component, we used population STR data from Spain reported by Camacho et al., (2007). It is also clear that all African genes originated in Western Africa. Therefore, we selected and pooled African STR data from two Western-Africa population studies: Angola (Beleza et al., 2004) and Guiné-Bissau (Goncalves et al., 2002). We preferred pooling two population data, because ethno-historical information is not precise about the slavery geographic migration origins. However, several genetic studies indicate that most Western African populations (including Angola and Guiné-Bissau) are very similar among them (Adeyemo et al., 2005). Our analysis and allele frequencies obtained in previous studies also confirmed this view, showing no significant differences between Angola and Guiné-Bissau populations (data not shown).

The Amerindian gene component is more complex and unclear; because of early extinction of most Amerindian tribes and ethno-historical records are incomplete. We hypothesized that because Ngöbe and Cueva groups were the major Amerindian tribes populating the Isthmus of Panama during European conquest, these two groups might also be the major Amerindians ancestors of the modern Panamanian mestizos. The contribution of each of the Amerindian groups to the genetic make up of Panama is unknown. Although the Ngöbe are characterized as Chibchan-speaking, the Cueva origin is not clear, since they are extinct. Some authors suggest that Cueva were Chocoan, though others consider them as Chibchan (Loewen, 1963, Constenla, 1991). To evaluate this question, we included in the analyses Ngöbe (Chibchan) and Embera (Chocoan) polymorphic short tandem repeat microsatellites data previously reported by us (Castro et al., 2007). We chose Emberas as a Chocoan Amerindian model to potentially indentify Cueva's genes because Embera are well characterized as Chocoan/Paezan-speaking and in the present they inhabit (approximately 31,284, according to NISC) part of the ancestral Cueva territory (Figure 1), in scattered areas from Colombia to Panama. Emberas have been genetically and linguistically associated with the Waunaan Amerindian group, which is another Chocoan/Paezan tribe that also inhabits in Panama and Colombia but genetically and linguistically separated from Chibchan-Kunas and even farther from the Ngöbe's language (Kolman and Bermingham, 1997; Constenla, 1991). The pre-Columbian geographical proximity of Cueva and Embera Amerindians could suggest that they had cultural and genetic exchange. Emberas (and Kunas) have moved to Darien in by successive westward migratory waves and their population is mainly focused in their reserved area: the Embera-Wounaan Comarca. Given their isolated life style and recent migration, Embera contact with Panamanian mestizos is very low. Both Ngöbe and Embera show less than 2% of admixture with mestizos or any other Amerindian tribes (Barrantes et al., 1990; Kolman et al., 1995) and also shown on Figure 2.

Data Analysis

After removal of samples/data from subjects not fulfilling the criteria of inclusion (mentioned above in mestizo volunteers selection), we considered for the analyses approximately 650 unrelated Panamanian mestizos. The software STRUCTURE (Pritchard et al., 2000; Falush et al., 2003) was used to estimate the admixture patterns and the structure of the total population and per provinces/regions. Other population parameters were calculated using PopGene, version 1.32 (available at http://www.ualberta.ca/~fyeh/). Observed and expected genotype frequencies were determined using two algorithms (Nei, 1973 and 1978) and the likelihood ratio (G-square) test was performed to estimate Hardy-Weinberg expectations per locus per province and the entire country. Allele frequencies were calculated, and we also computed, as measures of gene diversity, the effective number of alleles (Ne) (Hartl and Clark, 1989) the observed number of alleles (Na), and Shannon's information index (I) (Shannon and Weaver, 1949) per locus. F-statistics (FIT, FST and FIS) were calculated using two approaches (Hartl and Clark, 1989) and Weir (1990); for a three-level sampling hierarchy: individual, subpopulations (provinces) and overall entire population (the country). Gene flow was estimated as number of migrants (Nm) from FST (Slatkin and Barton,1989) for provinces. We computed unbiased genetic identity (Nei, 1972) and genetic distance (Nei, 1978) among

provinces and constructed a dendrogram based on Nei's genetic distances using UPGMA method with an adoption of program Neighbor of Phylip version 3.5c by Joe Felsenstein. The MS Excel workbook template PowerStatsV12 (Promega Corp.) was used to calculate forensic parameters: matching probability and power of discrimination. Paternity parameters were estimated too, including power of exclusion and typical paternity index.

RESULTS

Ancestral Gene Admixture and Population Structure

Ancestral estimates of genetic admixture are shown in Table 1. Modern Panamanians are a trihybrid group with contributions from African (24%), European (25%), and Ngöbe/Chibchan Amerindians (51%). Chibchan genes are distributed throughout the country, showing relatively high levels of contribution within all provinces but are mostly concentrated in Coclé (70%), Chiriquí (64%), Veraguas (50%), and Panama (50%), but in lesser amounts in Los Santos (33%) and Colón (34%). Colon province showed the highest proportion of African genes (47%), followed by Panama province (27%) and Los Santos (25%). The lowest amount of African genes was found in Coclé (10.2%) and Chiriquí (14.8%). Significant proportion of European genes were 42% in Los Santos, 40% Herrera and the lowest was in Colon (18.6%). Overall, Panama as country showed high genetic heterogeneity (86%). The most heterogeneous provinces were Panama and Colón with 85% and Veraguas with 82%. The least heterogeneous provinces were Coclé (70%) and Chiriquí (72%). Heterogeneity measurements for Herrera and Los Santos were 79% and 76%, respectively. Additionally, we performed multiple comparison analyses of variance, which showed significantly diverse patterns of genetic structure and admixture among all provinces, except between Herrera and Los Santos (Figure 2). The Embera/Chocoan group did not contribute to the genetic profile of current Panamanian population (only 2.6% in average).

Estimates of admixture were complemented by genetic distance analysis among provinces and were evidenced on a dendrogram based on information from STR loci (Figure 3). Two main conglomerates are shown, one including Veraguas, Panama, Chiriquí and Coclé and the other conglomerate comprising Herrera, Los Santos and Colon. Admixture patterns among provinces showed in Figure 2 are similar to the clustering distribution showed on the dendrogram, however, there are some differences subjected to consideration. Coclé, Chiriquí and Veraguas seem to be very similar in admixture/structure, but Veraguas grouped a little separated, though in the same conglomerate. Herrera and Los Santos showed no significant differences in genetic structure pattern, but Los Santos is joined to Colón whereas Herrera clustered a little apart though in the same group.

Allelic Frequency Variation

Allele frequencies of fifteen STR loci from total mestizo population of Panama are shown in Table 2. Importantly, there is missing data from some loci of some ancestral populations. However, because of the high percentage of Ngöbe/Chibchan genes in provinces such as Coclé, Chiriquí, and Veraguas, and the lack of information from some ancestral Amerindian loci, it is reasonable to hypothesize that many of those highest alleles in Panamanian mestizos came from Chibchans ancestors. For example, Table 3 shows the alleles with the highest frequency per province, overall country and from ancestral populations. For instance, data from ancestral Africans and Europeans at loci D5S818 show their highest allele frequencies in allele D5S818*12. In contrast, in most of Panamanian provinces, the most frequent allele is D5S818*11, probably implying that the D5S818*11 allele was acquired from ancestral Chibchans. Similar approach might be used to potentially infer Ngöbe ancestral missing data from D2S1338*23 and D18S51*14 alleles. Moreover, Table 3 shows that the highest alleles in Embera/Chocoan for TH01 and TPOX loci are *8 and *6, respectively, but Ngöbe showed *6 and *11, respectively for these loci. Moreover, ancestral popualtions from Africa and Spain showed highest frequencies at allele *7 and *9.3, respectively for these loci (TH01 and TPOX). However, all mestizo populations from Panama matched with Ngöbe TH01*6 as their highest allele. Similarly, TPOX*11 was the allele with highest frequency in all mestizo samples matching with Ngöbe and African as highest ancestral alleles in mestizo, but not with Embera/Chocoans *6. It is very unlikely that Embera/Chocoans contributed to those missing alleles given that they are genetically different from Ngöbe/Chibchan as shown in Figure 2, which is also supported by previous works with nuclear and mtDNA from those Amerindian populations (Kolman and Bermingham, 1997). This is discussed below in a comparative perspective of mestizo's Amerindian genes from autosomals (reported here) with mestizo's mtDNA from a previous report (Perego et al. 2012).

HWE, Genetic Diversity and F-Statistics

Loci heterozygosity, genetic variation and diversity statistics are shown in Table 3. Loci with the highest number of polymorphic alleles (Na) were D18S51 (20), D21S11 (19) and FGA (18), whereas the lowest polymorphic were at TH01, D13S317 and TPOX, all with 8 alleles. Loci with the best effective numbers of alleles (Ne) were found in D2S1338 (8.687) and D18S51 (8.433), but the minimums were for TPOX (3.405) and D3S1358 (3.503). The most diverse loci based on Shannon's Index (I) were 2.303 and 2.275 detected in D18S51 and D2S1338, respectively. We found the lowest Shannon's diversity in TPOX (1.442) and D3S1358 (1.468). Loci D18S51 (87.40%) and D2S1338 (87.30%) showed the highest heterozygosity but TPOX (69.20%) and D3S1358 (71.90%) displayed the lowest. HWE-Probability indicates that most loci are in accomplishment of equilibrium expectations, however, TH01 (0.269) and D2S1338 (0.123) probabilities were relatively low.

Genetic diversity was also addressed by using F-Statistics to evaluate subpopulation (provinces) diversity information. FIS -statistic values indicate high polymorphisms within each province, showing the most informative heterozygosity occurring in D21S11 (-0.049). All loci showed differences in their FST low values among provinces, indicating high polymorphisms. Similarly low FST values have been reported in other admixed populations in Latin America (Assis-Poiares et al., 2010; Rubi-Castellanos, et al., 2009). However, our dendrogram and genetic distances indicate important differences not detectable at the differentiation index FST. Consistent with FIS parameter values, FIT -statistics suggested high heterozygosity diversity in overall population, being also the most informative in D21S11 with -0.037. Although gene flow (Nm) values are probably more related to ancestral gene flow, rather than migration among provinces, it was estimated based on FST for provinces, showing the highest gene flow levels in vWA (45.680), D8S1179 (40.592) and D5S818 (38.000).

Forensic Genetic Parameters and Anthropological Implications

The short tandem repeats analyzed in this study are of importance for biomedical studies in forensic casework, forensic anthropology and paternity tests. The observed high genetic heterogeneity, polymorphisms and differences in admixture among provinces guided us to ask if these diversity levels might have relevance in the use of these markers in those anthropological applications. The high genetic polymorphisms and variation in diversity found may imply that the use of these loci require applying specific calculations for each province as sub-populations to validate results. We therefore, calculated informative parameters of relevance in forensic and paternity tests. We computed information parameters at each locus, per province and the country (Table 3). These data suggest that the most informative loci in forensic applications for Panamanian population are D18S51, FGA and D21S11. However, all other loci are very informative too, when used in combined analyses. Combined analysis of 15 STR loci in forensic parameters for matching probability (1 in 5.3x10^17) and power of discrimination (1.000), showed very significant informative discrimination in the country. Paternity parameters such as combined power of exclusion (0.999998992) and combined typical paternity index (924654.0396), also showed very informative statistics for the country. Similar results were found for each province, thus validating these loci as excellent markers in biomedical applications in forensic genetics (data not shown).

Inference on Medical Anthropology of Genetically Associated Diseases

Numerous epidemiologic studies indicate that gene ancestry is important when looking at groups instead of individuals (Rosenberg et al. 2005; Tang et al., 2005; Kittles and Weiss 2003). However, it is important to denote that gene ancestry itself is not the cause of diseases, because it involves several interactions such as genes, environment and lifestyles (Nebert et al., 2003; Ober and Vercelli 2011; Bravo et al., 2011; Scatliff et al., 2011). Therefore, genetic ancestry is a useful indirect marker to evaluate potential risk factors and disparities in some health-related phenotypes. For instance, it is described that during admixture and migratory processes in Panama, several diseases affected the population in a race-selective manner (Jaén Suárez, 1998). As a matter of fact, it is reported that many diseases vastly diminished Amerindians; while the Antillean (African-Caribbean) were the most resistant people to most infections (Poveda-Ramos, et al., 2004). Pharmacogenetic studies point out metabolic differences of Amerindian tribes relative to European and Africans result in drug biotransformation due to enzyme polymorphisms (Jorge et al., 1999; Jorge-Nebert et al., 2002; Jorge and Arias, 1995). Epidemiologic studies indicate high incidence of metabolic syndrome diseases such as lipid metabolism, and diabetes in Amerindians when they are moved to modern society lifestyles (Hanis et al., 1991;

Chakraborty and Weiss 1986; Gower et al., 2003; Torun et al., 2002; Klimentidis et al., 2009:). These phenotypes/diseases are highly inheritable and major risk factors for other complex diseases such as cardiovascular disease, strokes, and Cancers (Basu et al., 2009).

The observed heterogeneity and differences in ancestral proportions among provinces and the extensive epidemiological genetic data reported on the literature, led us to investigate the possible relationship of our gene admixture findings with regional differences in the incidence of deaths by diseases. Gathering National Government vital statistics information of the Panama's Ministry of Health from the last 20 years, we found that the two major causes of deaths by disease in the Republic of Panama are cerebrum/cardiovascular diseases and cancers. We focused our study in prostate cancer and cerebrum/cardiovascular disease. For prostate cancer, we collected data from 1985 to 1997 (After 1998, most cancer patients are moved to be treated in Panama city); and for cerebrum/cardiovascular diseases we collected data from 1998 to 2010. The incidence of these diseases was statistically analyzed using One-way ANOVA and compared among provinces. We determined significant differences in the incidence of diseases among provinces. Even more, we found out that the incidence of these diseases seems to correlate with the ancestry component of these provinces. Prostate Cancer showed significantly higher incidence in Colón and Panama, which are also provinces with the highest proportion of African genes (Figure 4A). Furthermore, cerebrum-strokes/cardiovascular diseases showed significantly higher incidence in Herrera and Los Santos provinces (Figure 4B), which are the provinces with the highest proportion of European admixture, but also with moderately high contribution of African genes.

DISCUSSION

Panamanian Mestizos are Heterogeneously Admixed and Highly Polymorphic

Genetic structure analyses based on 15 STR autosomal markers of the Panamanian population showed that it is highly polymorphic as indicated by their differences in admixture among individuals and provinces (Figure 2). Geographic distribution of Amerindian, African and European genes are consistent with ethno-historical data (Jaén-Suárez, 1978; 1998). Allelic patterns and diversity parameters showed in Tables 1 and 3 also summarize this pattern of genetic diversity among provinces. Genetic distances and dendrogram (Figure 3) also support these heterogeneous findings. F-Statistics, forensic and paternity parameters were all consistent with genetic diversity/heterogeneity parameters calculated for each loci and provinces. Forensic and Paternity parameters of 15 STR loci indicated that the information provided by these markers is highly significant and useful in forensic casework and anthropological applications in overall population and provinces. The population of Panama shows relatively high levels of contribution from all three ancestral populations: 24% African, 25% European and 51% Amerindian. These results differ from previous report from Arias et al. (2002), who reported 38% of African, 36% Amerindian and 25% European genes. Thus, our results might evidence the changes in population growth and migratory patterns in recent years, but also could be related with methodological differences of using only two genetic/classical systems versus 15 STR informative markers reported here. In contrast to Panama, most Latin American countries and cities are dominated by one or two genetic origins (Table 1). Costa Rica, Medellin City (Colombia) and Mexico City are mainly composed of Amerindians and Europeans genes. Uruguay, Buenos Aires (Argentina) and Santiago (Chile) are mostly composed of Europeans. African genes dominate Barbados and Jamaica.

Amerindian Genes are from Chibchan Origins

The Panamanian mestizos's Amerindian component is Ngöbe/Chibchan and that there is no significant contribution of Embera/Chocoan ancestry. Our analysis calculated 46% of Ngöbe/Chibchan and only 2.6% of Embera/Chocoan genes in average. Given the present and pre-Columbian geographic distribution of Ngöbe/Chibchan-speaking tribes, it is not surprising to find Chibchan genes in Coclé, Chiriquí and Veraguas provinces but Chibchan genes are also dominating Panama and Colon Provinces in the Eastern. However, given the uncertainty of data on Cueva Amerindians, who are sometimes classified as Chocoans and inhabited Panama and Colón provinces, our findings might imply that Cueva were Chibchan or simply they were unable to significantly contribute to the admixture process due to their early extinction. However, Arias et al. (1992) reported that an isolated mestizo group of Amerindian ascendance (Cholos of Cocle) from central mountains of Panama (Cocle Province) displayed both Ngöbe-Chibchan and Kuna-Chibchan markers. In particular, they found a remarkable frequency (0.52) in the allele variant Pepa-Kun of peptidase-

A, which is undetectable in Ngöbe and Chibchans from Western Panama (Barrantes et al., 1990). Pepa-Kun allele variant is found in high frequency in Eastern Kuna-Chibchan Amerindians (0.42) and Chibchan-speaking groups from Colombia (Arias et al., 1992; Barrantes et al., 1990). It is not viable that Kunas passed the Pepa-Kun allele in such high proportion to the Coclé's mestizos, because by the time that Spaniards arrived to Panama, Kunas were limited to the North-West Caribbean in Colombia, close to Chocoan Emberas (see maps). This view is also supported by previous report on mtDNA analysis of Panamanian mestizos, showing that 51% of Amerindian mtDNA corresponded to haplogroup A2, a very abundant haplogroup in Ngöbe and Kuna, but low in Embera/Chocoans (Perego et al. 2012). The same study also showed that Panamanian mestizos displayed low frequency of Embera/Chocoan-specific haplotypes. Nowadays, Kunas are spread out in the North-East Caribbean coast of Panama and Darien Provinces. Being highly endogamy and relatively isolated, their contact with mestizos is recent and has not significantly contributed to the admixture of mestizos. Therefore, this supports the possibility that the Cueva Amerindian were Chibchan and might have provided some fraction of Chibchan genes to Panama and Colón provinces. Furthermore, some authors have suggested that Cueva had linguistic affinity with Chibchan Kuna (Loukotka 1968; Greenberg, 1987), thus supporting Cueva's possible Chibchan origins and potential genetic affinity with Kunas. Mitochondrial and nuclear DNA analyses have also confirmed Chibchan genetic relationship between Kunas and Ngöbes (Kolman and Bermingham, 1997).

It is doubtful that Chibchan genes in Panama and Colón provinces are the result of recent migrations from central provinces during the construction of the railroad and the Panama Canal, because only 1% of workers were of local origin (Maloney, 1993). More recent migrations from central provinces to Panama City in the second half of the XX century are also reported, thus also carrying some Ngöbe/Chibchan genes to Panama Province, but most demographic data indicates that the modern population of Panama and Colón provinces has grown in situ since Colonial times (Jaén-Suárez 1998). Panama City was founded on the site of a Cueva Village in 1519, and the city incorporated proximal Amerindian villages, relief sites, and satellite smaller cities/towns. Accordingly, Panama City has taken population from Santa Maria del Darien, Acla, Nombre de Dios, Chepo, and other small cities/towns in Cueva's territory (Figure 1). For example, Santa Maria del Darien and Acla were respectively abandoned in 1524 and 1527, and all their populations, including Amerindians and mestizos, moved to Panama City. Additionally, numerous documentation from XVI and XVII centuries also mentioned that Amerindian villages were rapidly absorbed due to fast miscegenation, dispersion and deaths (Jaén-Suárez, 1998). Panama City and Portobelo (in Colon Province), the biggest cities of the isthmus absorbed most of these smaller cities and Amerindian villages. In the Colón Province, Colón City as we know it today was founded in 1852, but its population is the result from the fusion of Nombre de Dios, Portobelo, and other small towns and villages. Cueva people inhabited Panama and Colón provinces for about 30-40 years after the arrival of Europeans (Romoli 1987), probably enough time to pass some genes. Multiple sources support that many Amerindians integrated into the new European Colonial society, thus contributing even more to admixture (Torres de Arauz 1972; Castillero Calvo, 1995).

Distribution of African and European Genes are Consistent with Migratory Waves

Although the overall admixture of African and European genes was 24% and 25%, respectively, they are differently distributed and arrived in different waves within provinces. As mentioned above, ethno-historical data indicate that African genes arrived to Panama first in the 16th century directly from Western-Africa and whose descendants are nowadays named African-Hispanics. In the second half of 17th and beginning of 19th centuries, the second flow of African genes came from the Antilles, and whose descendants are called African-Caribbean. Our genetic data also support this view. Colón and Panama provinces showed the highest component of African genes. These two provinces were the major distribution places for African slaves traffic during the European Colony, but also the most important migration focus of Antillean (African-Caribbean) during the construction of the railroad and the Panama Canal. In 1911, Colón City Census showed 56% of African inhabitants (Jaén-Suárez, 1998). However, an important percentage of African genes were also found in all other provinces, probably acquired during slavery in Colonial times. A large number of African slaves escaped into the isthmus jungles (Arias etal. 2002). This is also consistent with the fact that Los Santos and Herrera also showed relatively high levels of African contribution, together with the highest levels of European genes suggesting a co-

migration pattern of Spaniards with their African slaves in those provinces. This is explained by the polygamy practices of Spaniards men with women slaves, thus constantly increased European genetic baggage together with African genes. In contrast, Colón province, having the highest amount of African genes, showed the lowest contribution of Europeans. The admixture pattern showed in Table 1 and observed in Figure 2, also confirm little variation in European genes, within all provinces, except Los Santos and Herrera, which are both very similar. These two provinces are not mountainous and were rapidly colonized, implying that Amerindians soon died or fled to remote areas in the mountains of Chiriquí, Bocas del Toro and Veraguas (Arias et al., 1992). African slaves replaced Amerindians, which explain low Amerindian contribution in Herrera and Los Santos compared to Chiriquí and Veraguas. Chiriquí showed the lowest amount of African genes, probably as a result of its inaccessibility in terms of mountainous topography and long distances by Colonial times (Arias et al., 2002).

Regional Incidence of Diseases is Comparable with Genetic Ancestry

The highly polymorphic patterns and differences in ancestral distribution among provinces made us to consider potential correlates with diseases. We focused on some of the major causes of death by disease per province/region and focused on prostate cancer and cerebrum/cardiovascular diseases. Interestingly, prostate cancer showed the highest incidence in provinces with the highest African ancestry (Colón and Panama) (Figure 4A). Extensive reports point out considerable disparities in prostate cancer risk with 60% higher incidence rate among African-Americans men compared with European-American men (Bock, et al., 2009; Zeigler-Johnson et al., 2008; Robbins et al., 2007). Importantly, due to their close proximity, most prostate cancer patients from Colón Province are usually treated in Hospital's facilities of Panama City, which may explain the slightly higher incidence in Panama, rather than in Colón. Cerebrum-strokes/cardiovascular diseases showed the highest incidence in the provinces with the highest proportion of European genes, together with moderately high proportion of African genes (Figure 4B). Brain strokes and heart disease risk factors include hypertension, diabetes, obesity, high cholesterol levels, which are health conditions consistently associated to European-American and African-American (Cheng et al., 2010; Hankey, 1999; Donnan et al., 2008). Moreover, heart disease and strokes are the primary cause of death for people of most ethnicities in the United States, being leaded by African-Americans, and European-American, respectively (U.S. National Vital Statistics reports, 2007; 2009, Heart Disease and Stroke Statistics 2011; the Centers for Disease Control and Prevention, Casper et al., 2003; Roger et al., 2011). Therefore, the highest incidence of Cerebrum-strokes/cardiovascular diseases in Los Santos and Herrera, with high European and African Ancestry, might imply a combined higher risk factor than in European and African alone.

The results obtained in this research provide evidence for how ethno-historical events shaped the geographic distribution of populations and our genetic analyses uncovered the genetic admixture differences. Moreover, cultural exchanges have modeled lifestyles that interact with genes leading to differences disease distribution that are comparable with genetic ancestry. Altogether, these result point out the anthropological approach of Panamanian mestizos as a model group to study complex health-related phenotypes. It also showed the potential occurrence of ancestry-environment interactions of gene-gene variants that might be rare in the ancestral populations as suggested the high European-African admixture in higher incidences of Cerebrum-strokes/cardiovascular diseases in Los Santos and Herrera. It also highlights the need of more cellular/in vitro models to study diseases toward the development of genomic medicine and improvement of healthcare polices in admixed populations, being Panama one of the most impressive examples of complex miscegenation processes.

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manuscript.

Table 1

Ancestral Gene Admixture Estimates per Province and Comparison with other Latin American Countries and Cities

	African:	European:	Amerindian
Veraguas	0.11	0.39	0.5
Panama Province	0.27	0.23	0.5
LosSantos	0.25	0.42	0.33
Herrera	0.2	0.4	0.4
Colón	0.47	0.19	0.34
Coclé	0.1	0.2	0.7
Chiriqui	0.15	0.21	0.64
Panama (Country)	0.24	0.25	0.51
Costa Rica [a]	0.09	0.61	0.3
Medellin (Colombia) [b]	0.09	0.66	0.25
Mexico City [c]	0.05	0.26	0.69
Barbados [d]	0.89	0.1	0.02
Jamaica [d]	0.84	0.12	0.03
Sao Paulo (Brazil) [e]	0.14	0.79	0.07
Puerto Rico [f]	0.21	0.64	0.15
Uruguay [g]	0.05	0.84	0.1
Santiago (Chile) [h]	0	0.57	0.43
Argentina [i]	0.025	0.78	0.194

[a] Moreno et al., 2003, [b] Wang et al., 2008, [c] Juarez-Cedillo et al., 2008, [d] Bena-Torres et al. 2008, [e] Ferreira et al., 2006, [f] Via et al., 2011, [g] Hidalgo et al 2005, [h] Sans, 2000, [i] Salzlin et al., 2007.

Table 2

Allele Frequencies of 15 STR Loci in Panamanian Mestizo Population.

ALLELE/ LOCUS	D8S1179	D21S11	D7S820	CSF1PO	D13S348	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D8S818	FGA
3						0.001									
5						0.002									
6						0.379		0.001				0.016		0.001	
7			0.007	0.014		0.248						0.008		0.050	
8	0.004		0.123	0.023		0.098	0.076	0.016				0.419		0.022	
9	0.006		0.081	0.017		0.136	0.179	0.151		0.002		0.089		0.116	
9.3						0.131									
10	0.056		0.262	0.228		0.005	0.075	0.154		0.002		0.039	0.005	0.056	
11	0.078		0.277	0.277			0.184	0.311		0.021	0.001	0.007	0.004	0.315	
11.2										0.001					
12	0.324		0.199	0.359	0.005		0.311	0.230		0.070		0.120	0.081	0.284	
12.2										0.024					
13	0.292		0.045	0.075	0.005		0.118	0.121	0.008	0.309	0.006	0.002	0.072	0.144	
13.2										0.087			0.002		
14	0.275		0.005	0.006	0.115		0.052	0.016		0.237	0.059		0.156	0.012	
14.2										0.052			0.001		
15	0.133		0.001	0.001	0.442		0.005	0.001		0.121	0.110		0.116	0.001	
15.2					0.001					0.059					
16	0.026				0.240				0.035	0.053	0.380		0.143		
16.2										0.017					
16.8										0.001					
17	0.006				0.119				0.133	0.002	0.254		0.190		
17.2										0.001					
18					0.070				0.041		0.116		0.078		0.004
18.2															0.006
19					0.005				0.117		0.060		0.057		0.066
19.2													0.001		
20									0.129		0.011		0.055		0.073
20.2															0.001
21			0.001					0.001	0.049		0.004		0.018		0.107
22								0.001	0.152				0.014		0.118
22.2															0.001
23									0.160				0.007		0.148
23.2															0.004
24									0.090				0.001		0.222
24.2		0.001													
25									0.058				0.001		0.150
26		0.002							0.015				0.001		0.073
27		0.010							0.002						0.019
28		0.100													0.007
29		0.207													0.002
30		0.274													
30.2		0.022													
31		0.098													
31.2		0.054													0.002
32		0.034													
32.2		0.122													
33		0.007													
33.2		0.050													
35.3		0.001													
34		0.001													
34.2		0.004													
35		0.012													
36		0.002													
39		0.001													
48.2															0.001

Table 3

Gene Variation and Diversity Parameters, and Comparisons of Alleles with the Highest* Frequencies Among Provinces and Ancestral Populations for 15 STR Loci.

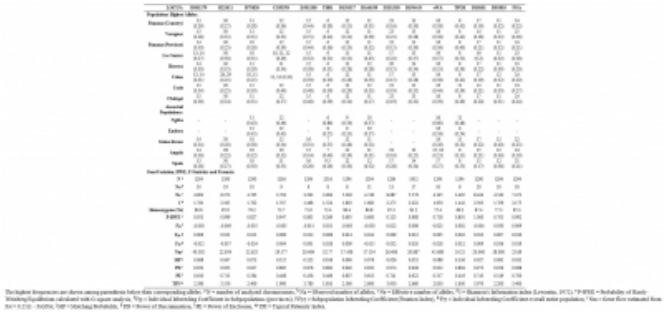


Figure 1

A. Pre-Columbian distribution of Chibchan and Chocoan Amerindians and major cities founded by Spaniards during colonial times. Most of these cities were founded in places originally occupied by Amerindians settlements. Some of these cities still exist and became in the main Capital City current Panamanian provinces. B. Present distribution of Amerindian tribes, and Panamanian provinces. These urban regions/City of provinces are the main areas where DNA samples were collected. This Map was adapted based on Barrantes et al., (1990), Arias et al., 1990, Romoli, (1987) and Jopling (1994).

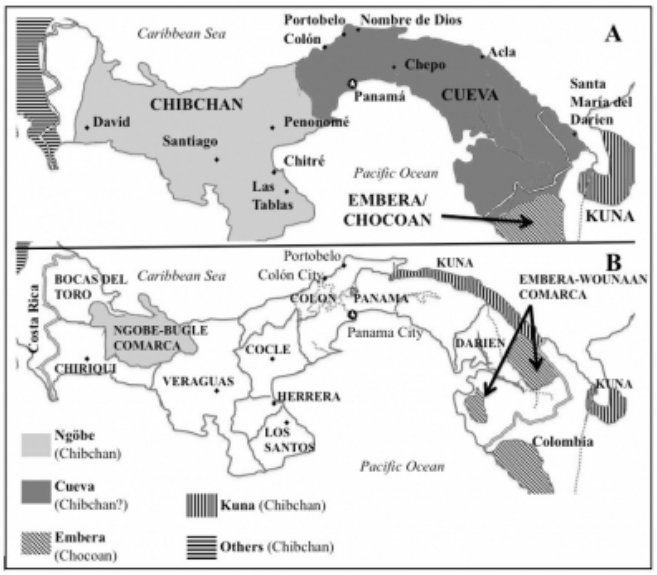


Figure 2

Genetic Structure and admixture of Panamanian Mestizos. Analyses were performed using STRUCTURE. Color codes blocks corresponds to ancestral population gene clusters. Each vertical line represents an individual subject ancestry proportions (top). Below, average ancestral contribution per province (bottom). Labels above bars indicate ancestral population and Panamanian provinces.

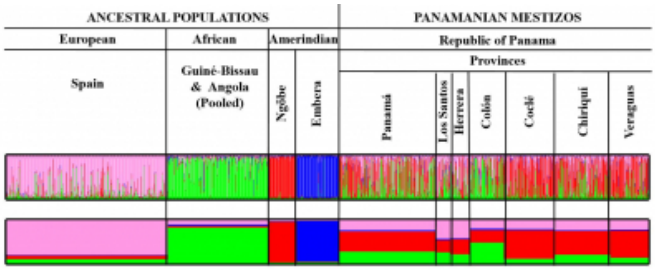


Figure 3

Dendrogram Based on Nei's (1978) Genetic distance constructed by UPGMA algorithm method estimated from 15 STR loci information of 7 provinces from Panama.

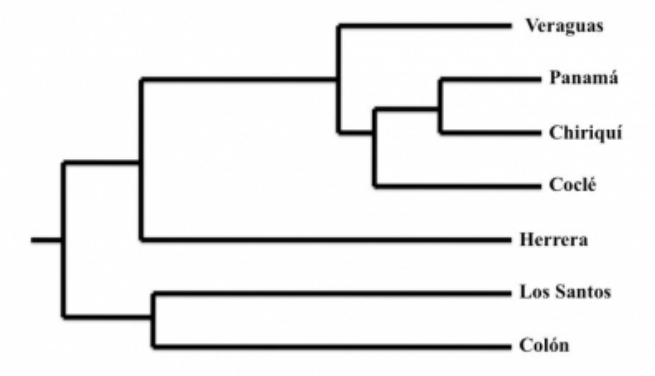
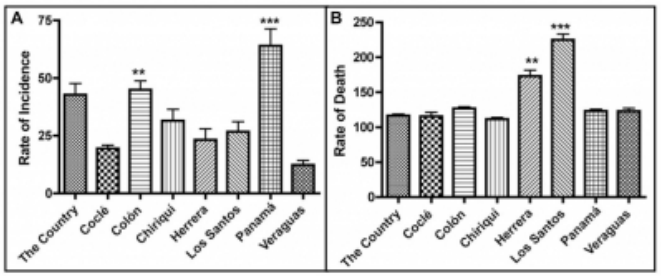


Figure 4

A. Incidence of prostate cancer per province based on 100,000 male inhabitants 1985- 1997. One-way ANOVA and Bartlett's statistic post-correction determined that provinces with highest proportion of African genes (47 % Colon and 27% Panama) showed the highest incidence of prostate cancer compared with all other provinces with lower African admixture ($P < 0.001$ for Panama and $P < 0.01$ for Colon). B. Deaths caused by brain strokes and heart disease per province based on a rate of 100,000 inhabitants 1998-2010. One-way ANOVA and Bartlett's statistic post-correction showed that Los Santos and Herrera, provinces with the highest proportion of European genes (42 and 40%, respectively) and moderately high African genes (25% and 20%, respectively) also displayed the highest rate of deaths by brain strokes and heart diseases compared to all other provinces showing lower proportion of European admixture ($P < 0.001$ for both provinces).



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