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African genetic ancestry is associated with a protective effect on Dengue severity in colombian populations

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ABSTRACT

The wide variation in severity displayed during Dengue Virus (DENV) infection may be influenced by host susceptibility. In several epidemiological approaches, differences in disease outcomes have been found between some ethnic groups, suggesting that human genetic background has an important role in disease severity. In the Caribbean, It has been reported that populations of African descent present considerable less frequency of severe forms compared with Mestizo and White self-reported groups. Admixed populations offer advantages for genetic epidemiology studies due to variation and distribution of alleles, such as those involved in disease susceptibility, as well to provide explanations of individual variability in clinical outcomes. The current study analysed three Colombian populations, which like most of Latin American populations, are made up of the product of complex admixture processes between European, Native American and African ancestors; having as a main goal to assess the effect of genetic ancestry, estimated with 30 Ancestry Informative Markers (AIMs), on DENV infection severity. We found that African ancestry has a protective effect against severe outcomes under several systems of clinical classification: Severe Dengue (OR: 0.963 for every 1% increase in African ancestry, 95% confidence interval (0.934–0.993), *p*-value: 0.016), Dengue Haemorrhagic Fever (OR: 0.969, 95% CI (0.947–0.991), *p*-value: 0.006), and occurrence of haemorrhages (OR: 0.971, 95% CI (0.952–0.989), *p*-value: 0.002). Conversely, decrease from 100% to 0% African ancestry significantly increases the chance of severe outcomes: OR is 44-fold for Severe Dengue, 24-fold for Dengue Haemorrhagic Fever, and 20-fold for occurrence of haemorrhages. Furthermore, several warning signs also showed statistically significant association given more evidences in specific stages of DENV infection. These results provide consistent evidence in order to infer statistical models providing a framework for future genetic epidemiology and clinical studies.

1. Introduction

Transmission and severity of infectious diseases are the result of a complex interaction between environmental and biological factors (Tibayrenc, 2007). A major feature of most human infections is their considerable phenotypic variability, ranging from asymptomatic to lethal (Casanova and Abel, 2007; Chapman and Hill, 2012). Recently, the finding of novel genetic variants involved in immune response to several pathogens allowed to emphasise the role of shared host signalling pathways in the pathogenesis of infectious diseases, providing important insights into the genetic regulation of such response in humans (Chapman and Hill, 2012).

Substantial evidence revealed that Dengue Virus (DENV) infection is largely mediated by immunopathological mechanisms, which are major determinants of variation in disease outcomes (Coffey et al., 2009), suggesting that variants in genes associated with immune response must be carefully assessed (Sierra et al., 2007a).

Regarding to that issue, several Single Nucleotide Polymorphisms (SNPs) from molecules implicated on different infection moments and severity spectrum stages have been associated with protection and susceptibility to severe forms (Coffey et al., 2009). In 2011 the first Genome-Wide Association Study (GWAS) in Dengue was performed in a Vietnamese cohort including Dengue Shock Syndrome (DSS) cases, finding only two associated genes

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which had never been reported (Khor et al., 2011). Table A1 (Supplementary material) contains detailed information about known variants associated to Dengue.

However, the allele frequencies in some of these SNPs display substantial differences between different continental populations (Supplementary material, Table A2), driving to biases in the analyses due to stratification created by population structure patterns and genetic admixture events (Balding, 2006), such structure become an important confounder covariate in regression analyses and must be addressed in detail.

According to the fact that human populations have significant differences in their genetic makeup (Wang et al., 2008), they would be expected to exhibit different patterns of susceptibility/resistance to infectious diseases (Tibayrenc, 2007). Epidemiological studies, such as those conducted in Africa and the Caribbean have suggested that populations with African ancestry may be less susceptible to DENV disease severe forms (Herrero et al., 2013).

For the African continent, in spite of having suitable conditions and intense circulation of DENV serotypes, there are no reports of severe cases (Were, 2012), as well as in Haiti, according to information collected between 1994 and 1999 (Halstead et al., 2001). During several outbreaks in Cuba between 1981 and 2001 it was found that individuals classified as Black by self-reported ethnicity data presented considerably less cases of Dengue Haemorrhagic Fever (DHF) and DSS than Mestizo and White groups (Sierra et al., 2007a). Furthermore, a Brazilian sample was analysed with genetic ancestry estimates and also showed a significant protective effect of the African component (Blanton et al., 2008).

The ancestral genetic background of Latin American populations (such as Colombia) has been shaped by recent and complex admixture processes between European, Native American and African populations (Bedoya et al., 2006; Rojas et al., 2010; Vergara et al., 2013; Wang et al., 2008). Such context is particularly interesting regarding the study of genetic epidemiology and the variation and distribution of alleles involved in susceptibility to complex diseases (Rojas et al., 2010). Studying admixed populations offers a novel way to investigate the pathogenesis of infectious diseases and to identify genes playing a causal role in modulating human susceptibility and/or resistance to infectious pathogens (Ettinger et al., 2009).

Our collaborators from ICMT previously found higher frequencies of DHF, thrombocytopenia and IL6 serum levels in self-reported mestizo individuals compared to self-reported Afrodescendants in populations at north-western Colombia (Restrepo et al., 2008), being the starting point of the present study. Having all this supporting evidence we aimed to assess the effect of genetic ancestry on susceptibility to Dengue severity according to several classification systems in three north-western Colombian population samples using autosomal Ancestry Informative Markers (AIMs) to infer the individual percentages of European, African and Native American Ancestry components.

2. Methods

2.1. Sample and clinical profiling

Two hundred-eighty-seven (287) unrelated individuals with confirmed DENV infection were recruited from Antioquia and Chocó Departments in Colombia during several outbreaks taking place between 2006 and 2012. Antioquia's samples were taken in two sub-regions: Valle de Aburrá (Here defined as Medellín Metropolitan Zone (MMZ)) and Urabá. After patients gave written informed consent, peripheral blood was taken by ICMT certified staff, who also did the clinical profiling of the sample.

Individuals that showed several basic symptoms related to DENV infection (fever, severe headache, retro-orbital pain, myalgia, arthralgia, nausea and vomiting) were tested by specific DENV IgM/IgG Enzyme-Linked Immunosorbent Assays (ELISA) (PanBio Dengue kit-Inverness Medical). Participants with confirmed infection were classified according to WHO clinical and laboratory criteria established in 1997 (WHO, 1997) and 2009 (WHO, 2009).

All the clinical tests suggested by WHO guidelines known as warning signs were performed in order to classify the patients according to both systems: (i) WHO (2009): Dengue without warning signs (D − WS), Dengue with warning signs (D + WS) and Severe Dengue (SD). (ii) WHO (1997): Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF). Furthermore, in addition, an Alternative Classification (iii) widely used on literature (Bandyopadhyay et al., 2006) was established based on WHO (1997) taking absence/presence of haemorrhages into consideration: Dengue Fever (DF), Dengue Fever with haemorrhagic manifestations (DF + H) and Dengue Haemorrhagic Fever (DHF).

Two recognised confounding factors were characterised: Occurrence of secondary infection that was established using the information of IgM/IgG titles obtained in the specific DENV ELISA performed, and Self-reported Ethnicity, classifying the participants as Afrodescendants or Mestizos.

2.2. AIM's selection and genotyping

DNA was extracted from 5 ml peripheral blood samples by Phenol–Chloroform method (Sambrook and Russell, 2006). 30 autosomal Ancestry Informative Markers (AIMs) widely distributed across the genome were selected based on previous studies where they were used (Parra et al., 2004, 1998) and according to their significant differences in allele frequencies in every ancestral population, showing differences higher than 40% at least between two populations (Supplementary Table A4), enhancing statistical power for genetic ancestry inference and estimation of individual percentages (McKeigue, 1997).

AIMs were typified via RFLP-PCR and INDEL methodologies, some of them using an ABI Prism 310 Genetic Analyser (Perkin Elmer – Applied Biosystems). See detailed conditions on Supplementary Table A3.

2.3. Genetic ancestry estimation

Allele frequencies and Hardy–Weinberg equilibrium were calculated with GENEPOP (Rousset, 2008) Ancestry proportions were estimated modelling the sample with the prior distribution of allele frequencies data from continental populations (European, Native American and African) in order to get a posterior distribution of the ancestral contribution in each sample, using the software ADMIXMAP (McKeigue et al., 2000). This software runs under Bayesian methodology inverting conditional probabilities merging genotypes at every locus for each sample with prior data from the reference samples in order to get the posterior distribution of each ancestry component for all participants. Ancestry values are recorded as percentages, with the three ancestry components always adding up to 100% for any participant.

2.4. Analysis

Logistic Regression was used in order to assess the effect of genetic ancestry on disease outcomes and warning signs while taking into consideration the following covariates: age (as a quantitative variable), sex (male = 0, female = 1), type of infection (primary = 0, secondary = 1), department and sub-region due their status of confounding factors. Since our samples are collected from two departments, Antioquia and Chocó, an indicator variable was