

Genetics and anthropology in studies on aging and Chagas disease

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Complex traits are difficult to disentangle, as the phenotypes result from the interaction of different basic components where genetics interacts with environment, epigenetics and stochasticity (Cevenini *et al.*, 2008). Studies in humans represent a particular challenge, owing to the pervasive and profound influence of cultural habits and specific evolutionary history on the different human populations. We will discuss the importance of adopting an integrated and multidisciplinary approach in genetic studies on two complex traits, such as aging and Chagas disease, as the most appropriate and correct methodology of data collection and analysis.

Longevity

Longevity is a very complex trait (Leroi *et al.*, 2005; Kirkwood *et al.*, 2005), and the phenotype of long-living people is not easy to define (Passarino *et al.*, 2007) The genetics of longevity has peculiar

and unexpected characteristics such as allele timing (Bonafè *et al.*, 2004; Invidia *et al.*, 2009) increased homozygosity at several loci (Bonafè *et al.*, 2001a; Cardelli *et al.*, 2008) gender prevalence (Passarino *et al.*, 2002) and specificity (Bonafè *et al.*, 2001b), strong dependence on the demographic structure (Yashin *et al.*, 1999; Passarino *et al.*, 2002; Cardelli *et al.*, 2008) and epidemiological specificities of the different populations, likely resulting from its post-reproductive occurrence (De Benedictis & Franceschi, 2006), which in turn suggests a minor or negligible role of natural selection. Thus, the genetics of longevity can be highly population-specific, requires a large number of subjects (Lescai *et al.*, 2009a) and different results in different populations are not unexpected and cannot be taken, at first glance, as a simple lack of reproducibility and validation failure (Cellini *et al.*, 2005). The data so far collected indicate that a variety of genes are involved (Franceschi *et al.*, 2005; Franceschi *et al.*, 2007a; Salvioli *et al.*, 2006; Capri *et al.*, 2008) but it interesting to note that those involved in

major pathways and functions such as IGF1/insulin pathway (Bonafè *et al.*, 2003), inflammation and immune responses (Carrieri *et al.*, 2004; Franceschi *et al.*, 2005), oxidative stress and lipid metabolism (Salvioli *et al.*, 2005; Lescai *et al.*, 2009b) and sirtuins (Bellizzi *et al.*, 2005) are well represented. Thus the genetics of longevity requires *ad hoc* study design which are not easy to perform in different populations (Franceschi *et al.*, 2007; De Rango *et al.*, 2008)

We will report our experience regarding two studies which have peculiar and unique characteristics, *i.e.*:

1. The AKEA study of exceptional longevity in Sardinia (Deiana *et al.*, 1999). In order to identify the centenarians all over the island and to collect data (health status, genealogical data) and biological samples (blood) a complex logistical organization was set up. We will review some of the major data we have collected regarding the prevalence of centenarians and their sex ratio and geographical clustering, as well as their phenotype and genotype (Passarino *et al.*, 2001; Deiana *et al.*, 2002; Carru *et al.*, 2003; Lio *et al.*, 2003; Poulain *et al.*, 2004; Pes *et al.*, 2004; Caselli *et al.*, 2006; Polidori *et al.*, 2007; Scola *et al.*, 2008).

2. The GEHA (Genetics of Healthy Aging) Project (2004-2010) in Europe (Franceschi *et al.*, 2007b). The aim of the project is to identify genes and gene variants involved in human longevity and healthy aging, with particular attention to the cross-talk between the nuclear and the mitochondrial genomes. To this aim, within the framework of this European Union (EU) project, 2500 old sibpairs (both sibs being 90+) and the same number of sex- and ethnicity-matched younger controls have been recruited in 11 European countries. The genome wide linkage analysis on the 90+ sibpairs has been completed and a GWAS study in the oldest members of the 90+ sibpairs is in progress. Moreover, mtDNA haplogroups and sub-haplogroups have been analysed in all the recruited people, in order

to confirm in a large sample of subjects derived from different geographical areas previous results suggesting a correlation between human longevity and mtDNA variants, both germ-line inherited and somatically acquired (De Benedictis *et al.*, 1999; Zhang *et al.*, 2003; Rose *et al.*, 2007), which appears to be population-specific (Dato *et al.*, 2004). Indeed, such studies on mtDNA variants at the population level may be biased by a variety of methodological issues (Santoro *et al.*, 2006; Raule *et al.*, 2007; Salvioli *et al.*, 2008).

In both studies, even if at a different scale, major logistical, ethical and genetical problems related to the complexity of the populations involved, emerged, stressing the necessity of a more comprehensive and integrative approach, capable of including demographers and historical demographers, anthropologists and local historians, besides geneticists, population geneticists and biogerontologists, for the best and more correct interpretation of the genetic results.

Chagas disease

Chagas disease affects several millions individuals in South America, where the *Trypanosoma cruzi* is endemic and causes higher mortality than any other parasite. Its main vector is the haematophagous *Triatoma infestans*. Clinical manifestations range from asymptomatic infections to potentially fatal cardiopathy. Rural populations are the most affected, due to housing conditions and underlying poverty and low education. Recently, cases of infected subjects have been reported in USA and Europe as a result of the massive migration from South America, arising problems for blood donors, organ transplantations and new births. The aim of our study was to test the hypothesis of a different genetic susceptibility to the *Trypanosoma cruzi* infection and its clinical outcomes, in two populations, such as the Wichí (Amerindian natives) and the Criollos (a complex admixture of people of European and native origin), living in the same geographic region

of Argentinian Gran Chaco (Mission Nueva Pompeya) where the infection is endemic (about 60-70% in both populations), but having different cultural habits and lifestyles as well as different historical-genetic-demographic characteristics. A critical issue in such field studies is represented by *the cultural and anthropological mismatch* between the native populations and the researchers coming from outside. Indeed, the anthropologists working since several years with the Wichì and Criollos in Mission Nueva Pompeya were fully aware that the conceptualization of diseases and particularly that regarding Chagas disease was substantially different from the one adopted by the geneticists and medical doctors. To this regard, we would like to stress the critical importance of the strict collaboration between the anthropologists, the medical doctors and the population geneticists which allowed us to overcome the non trivial and usually neglected difficulties (cultural and logistical) related to the approval of the Ethical Committees in Bologna and in Argentina, the signature of the informed consent and the collection of biological samples. Indeed, this study has been possible owing to the full involvement of the native populations and the reciprocal respect between the Wichì and Criollos and the anthropologists, resulting from their previous careful field studies. It is worth mentioning that the anthropologists devoted a particular attention to the preparation of a bilingual (Wichì and Spanish) booklets aiming to explain to the two communities the characteristics of the Chagas disease as well the implications of their participation to the study, taking into account their cultural characteristics.

Within this scenario, a preliminary step was to assess the genetic admixture of the populations under study, taking into account cultural/anthropological and socio/economical variables. To this aim a total of 600 individuals from the two populations was analysed With two sets of uniparental markers (mtDNA and Y chromosome) in order to estimate the degree of admixture and parental contributions in both populations (Yang Yao *et al.*, 2009). We characterised 17 STRs loci and

16 SNPs for the Y chromosome and the mtDNA haplogroups by sequencing the entire Control Region (D-loop) followed by RFLP analysis of the coding region. The Y chromosome SNPs definition shows a typical, but very high, prevalence of Amerindian haplogroups in Wichì, while in Criollos almost the same percentage represents European (admixture analyses highlight the main role of Spain and Italy) and Amerindian contribution. The mtDNA haplogroup analysis revealed an Amerindian origin for both populations, with the presence of European haplogroups only in <2% of Criollos. These results shed lights on the extreme admixture of the analysed populations and may help in explaining the distribution of Chagas morbidity in the Gran Chaco region. In addition, we are assessing the patterns of seropositivity in specific familiar groups in relation to their different ethnic origin (Wichì and Criollos), and are studying polymorphisms of candidate autosomal genes, to unravel the genetic basis of Chagas susceptibility.

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