23 Integrated Genetic Epidemiology of Chagas Disease

Michel Tibayrenc[1,*], *Jenny Telleria*[1], *Patricio Diosque*[2], *Juan Carlos Dib*[3] and *Christian Barnabé*[1]

[1]Institut de Recherche pour le Développement (IRD)/Centre National de la Recherche Scientifique (CNRS), Montpellier, France, [2]CONICET, Argentina, University of Salta, Salta, Argentina, [3]University of Antioquia, Antioquia, Colombia

23.1 What Is Integrated Genetic Epidemiology?

As authoritatively illustrated by this book, the impressive progress of molecular megatechnologies (high-throughput sequencing, microarrays, postgenomics) and the concomitant development of bioinformatics have considerably improved our knowledge on infectious diseases. However, there is a strong tendency toward compartmentalization in the research effort: scientists working on human (and other hosts) genetic susceptibility to infectious diseases are generally not aware of research on the role played by pathogens, and vectors in the case of vector-borne diseases. This results in each community of scientists tending to overemphasize the role of its study material. This compartmentalization is all the more distressing since coevolution between hosts, pathogens, and vectors should be considered a unique biological phenomenon. The term "integrated genetic epidemiology" (Tibayrenc, 1998a) has been coined to designate the approach consisting in simultaneously analyzing the impact of the host's, the pathogen's, and the vector's genetic diversity on the transmission and severity of infectious diseases as well as the coevolution processes between the three. The present chapter aims to show that Chagas disease is an excellent model to develop this approach. It briefly summarizes what is presently known about: (i) human genetic susceptibility to Chagas disease, (ii) the vectors' species and population diversity, and (iii) the parasite's genetics and evolution. Then it demonstrates how these three components could be merged in a unique approach.

*E-mail: michel.tibayrenc@ird.fr

Genetics and Evolution of Infectious Diseases. DOI: 10.1016/B978-0-12-384890-1.00023-6

23.2 Chagas Disease: A Major Health Problem in Latin America and Other Countries

Chagas disease remains by far the most serious health problem in Latin America. Control of the disease has been improved, but several million people remain at risk or are stricken by the disease.

From a clinical point of view, Chagas disease is a very serious illness. After infection by the parasite (see Section 23.3), patients develop an acute phase, which actually corresponds to parasitic septicemia. Mortality at this stage is approximately 5%. After a few weeks, patients who survive enter the indeterminate phase, with no symptoms. About 70% of patients will never exhibit any symptoms again. However, 30% of them will develop symptomatic Chagas disease. The most worrisome symptom is Chagasic cardiopathy, which leads to a severe cardiac insufficiency. Other clinical forms involve the digestive system (megacolon, megaesophagus) and cause severe functional abnormalities.

The health problem of Chagas disease is worsened by its being a "neglected disease" according to official classifications. As for the infectious diseases predominant in the southern world, malaria, AIDS, and tuberculosis receive special attention from WHO and other international health authorities, while other diseases tend to be underprioritized. However, the dispersion of Chagas disease from Latin America to nonendemic countries by population movements has started to create new epidemiological, economic, social, and political challenges as *T. cruzi* has spread throughout the world (Rodrigues Coura and Albajar Viñas, 2010). In the domain of scientific research, it is notable that the scientific community involved in Chagas disease research, although very productive, is tiny, but hopefully will expand.

23.3 The Chagas Disease Cycle

The Chagas disease cycle will be only briefly summarized here, since this chapter is not intended to be an exhaustive review of what Chagas disease is, but rather attempts to explain why this disease is a valuable model for integrated genetic epidemiology.

The causative agent of Chagas disease is a parasitic protozoan of the family Kinetoplastidae, which also includes *Trypanosoma brucei*, the agent of sleeping sickness (African trypanosomiasis) and the *Leishmania*, agents of the various forms of leishmaniosis.

Trypanosoma cruzi is transmitted by "true" bugs, heteropterous insects of the family Reduviidae, subfamily Triatominae. This subfamily has specialized in obligatory blood-feeding. It is worth noting that Chagas vectors include many different species and three principal genera, namely *Triatoma*, *Rhodnius*, and *Panstrongylus*. Vectors are infected by ingesting blood that contains the parasite. They transmit the parasite, not through their biting, but by their feces, which contains the infecting forms. Most vector species present the particularity of depositing feces while

they feed on their host. The parasite enters the host by excoriations, through the mucosa, even through intact skin.

Hosts comprise virtually all mammalian species, either domestic or selvatic, including of course humans.

23.4 Host Genetic Susceptibility to Chagas Disease

This section emphasizes what is known about the human species. Since Chagas disease strikes virtually all mammalian species, a general view of the role played by all these hosts' genetic diversity in the disease is difficult.

Human genetic susceptibility to Chagas disease is less well known than for other transmissible diseases such as Hepatitis C (Alric et al., 1999), tuberculosis (Bellamy et al., 2000), malaria (Garcia et al., 1998), AIDS (Dean et al., 1996), leprosy (Abel et al., 1998), schistosomiasis (Dessein et al., 1999), or visceral leishmaniosis (Bucheton et al., 2002).

Chagas disease could be a fine target for studying human genetic susceptibility. As for leprosy, the chagasic **phenotypes** are well defined: asymptomatic (ASY) versus symptomatic in the acute and chronic phases, and for chronic symptomatic Chagas, cardiac, digestive, and cardiodigestive manifestations. One can also distinguish between negative serology (Brenière et al., 1984) and positive serology in the chronic phase. Therefore, one can analyze not only genetic susceptibility to Chagas, in general, but also to its different clinical manifestations.

Another favorable situation is that Chagas disease strikes populations that are ethnically very diverse. Latin Americans have European, African, Amerindian, and mixed ancestries. Ethnic diversity is a parameter to take into account when exploring human genetic susceptibility to infectious diseases (Tibayrenc, 2007).

It is distressing that few people have considered these favorable features (clearly defined clinical phenotypes and ethnic diversity) to analyze human genetic susceptibility to Chagas disease. When looking for genes of susceptibility to transmissible diseases, two main approaches are available: (i) linkage studies and (ii) the candidate gene approach (Tibayrenc, 2007). Linkage studies are based on the analysis of pedigrees and families where some individuals have the studied disease while other individuals are used as controls. The genomes are screened with a high number of genetic markers (microsatellites or single-nucleotide polymorphisms, SNPs). If some markers happen to be statistically linked to the clinical phenotype surveyed (linkage disequilibrium), the genomic region where they are located is more finely dissected to look for the putative susceptibility genes. Candidate gene studies analyze genes that are putatively associated with the pathology surveyed. The reasons for inferring that a gene can be a candidate are either that it pertains to a genetic system frequently involved in susceptibility to infectious diseases (cytokine genes, HLA genes) or that it has proved to have such a role in animal experiments.

Genome-wide association studies (GWAS; Pennisi, 2007) are a more recent approach that screens the whole genome of thousands of individuals.

Research on human genetic susceptibility to Chagas disease has most often been based on the candidate gene approach and has chiefly, but not exclusively, explored the HLA system.

23.4.1 Genetic Heritability of Some Chagasic Characteristics

Pedigree analyses have shown an apparent heritability of some serological parameters. Chagasic seropositivity heritability in Brazil is estimated to be 0.556, which is high (Williams-Blangero et al., 1997). In the same country, the levels of IgA and IgG show a heritability of 0.33 (Barbossa et al., 1981). Zicker et al. (1990) have postulated a putative familial component in chagasic cardiopathy. This result has been challenged by Morini et al. (1994).

23.4.2 Role of the HLA System

As noted above, a great deal of the results on human genetic susceptibility to Chagas disease, especially the most recent ones, deal with the putative role of the HLA system. It has now been determined that the HLA supergene complex of several genes having a related role plays an important role in the transmission, severity, and clinical diversity of Chagas disease.

Apt (1988) surveyed the distribution of HLA antigens in 124 Chilean seropositive patients, divided into patients with chronic Chagas cardiomyopathy (CCC) and ASY patients.

Fernandes-Mestre et al. (1998) evidenced a lowered frequency of DQB1*0303 and DRB1*14 in chagasic patients compared with controls, suggesting independent protective effects to the chronic infection in this population. There was also a higher frequency of DRB1*01, DRB1*08, and DQB1*0501 and a lower frequency of DRB1*1501 in the CCC patients.

Layrisse et al. (2000) surveyed 113 seropositive patients (CCC versus ASY). They postulated that the HLA-C*03 allele constituted a risk factor for CCC.

Nieto et al. (2000) surveyed 172 Peruvian patients (85 seropositive with ASY = 52, CCC = 33; 87 seropositive controls) for the variability of the HAL-DRB1 and DQB1 genes. They recorded no allelic frequency differences between CCC and ASY patients. On the other hand, the DRB1*14-DQB1*0301 haplotype was statistically linked to seronegativity, which suggests that this haplotype has a protective role against Chagas disease.

Faé et al. (2000) were unable to find any significant relation between HLA and Chagas disease. This negative result has been challenged by many later studies.

Visentainer et al. (2002) surveyed 35 CCC patients with 72 control patients in Brazil. They found a statistically significant relation between CCC and HLA-DR2.

Moreno et al. (2004) surveyed 104 seropositive patients and 60 seronegative controls. They observed significant allelic frequency differences between seropositive patients and controls at the loci HLA D6S291 and IL-10. These results suggested epistasis between the HLA and IL loci that could be linked to susceptibility to Chagas disease.

Cruz-Robles et al. (2004) conducted a survey of 193 Mexican patients, 66 of whom were seropositive (either CCC or ASY) and 127 were seronegative controls. The results suggested that HLA alleles are associated with chronic Chagas disease and CCC. HLA-DR4 and HLA-B39 alleles could be associated directly with the infection by *Trypanosoma cruzi*, whereas HLA-DR16 might be a marker of susceptibility to CCC and HLA-A68 could be protective against CCC.

Ramasawmy et al. (2006a) analyzed the variants of BAT1, a putative anti-inflammatory gene (situated in the HLA class III region) in 76 ASY and 154 CCC patients, all seropositive. They found that some BAT1 could be used to predict the occurrence of CCC.

The same authors (Ramasawmy et al., 2008) analyzed the variants in the promoter region of the IKBL/NFKBIL-1 gene, which pertains to the MHC class I region. A total of 245 patients (76 ASY and 169 CCC) were surveyed. Subjects that were homozygous for the − 62A allele, had threefold risk of having CCC compared with those having the TT genotype. Moreover, the haplotype − 262A-62A was prevalent in CCC patients.

Borrs et al. (2009) surveyed 152 Argentinean subjects (71 seropositive individuals and 81 controls) for the variability of the second exon of HLA-DRB1.The DRB1*1103 allele was predominant in the control patients, which suggests that this allele plays a protective role. On the other hand, DRB1*0409 and DRB1*1503 had a significantly higher frequency in seropositive patients, while CCC subjects had a higher DRB1*1503 frequency.

23.4.3 Other Genetic Systems Involved in Susceptibility to Chagas Disease

Calzada et al. (2001a) surveyed 85 seropositive patients (53 ASY and 32 CCC) and 87 seronegative controls. A relation was found between the CCR5 59029 promoter polymorphism and susceptibility to CCC.

The same authors (Calzada et al., 2001b) surveyed 168 Peruvian patients (83 seropositive with 51 ASY and 32 CCC; 85 seronegative controls) for the variability of the natural resistance-associated macrophage protein-1 (NRAMP1) gene. No differences were observed between: (i) seropositive and seronegative subjects and (ii) CCC versus ASY patients.

Messias-Reason et al. (2003) surveyed 100 seropositive individuals (43 ASY and 57 CCC) and 100 seronegative control patients. They observed a positive relation between CCC and complement C3 and BF allotypes and a negative link between CCC and seropositive patients and the BFS haplotype. No significant associations were observed for the C3, BF, CAA, CAB, and C2 haplotypes.

Flórez et al. (2006) analyzed the relations between the IL-1A, IL-1B, and IL-1RN gene variability and Chagas disease in 260 seropositive Colombian patients (130 ASY and 130 CCC). They evidenced that the presence of the IL1-B + 5810G allele was associated with a higher CCC risk.

Ramasawmy et al. (2006b) analyzed the monocyte chemoattractant protein-1 (CCL2/MCP-1) gene variability in 245 seropositive patients (76 ASY and 169

CCC). They observed that patients harboring the CCL2-2518AA genotype had a fourfold higher CCC risk.

Drigo et al. (2007) surveyed 246 patients (80 ASY and 166 CCC). They found no link between pathology and tumor necrosis factor-α polymorphisms. This result contradicted a previous study from the same authors (Drigo et al., 2006), which showed that CCC patients having the TNF2 or TNFa2 alleles have a significantly shorter survival time compared to patients who have other alleles.

Similarly, Campelo et al. (2007) evidenced that the TNFa2, TNFa7, TNFa8, TNFb2, TNFb4, TNFd5, TNFd7, and TNFe2 alleles were overrepresented, whereas the TNFb7 and TNFd3 alleles were underrepresented in 162 Chagasic patients compared with 221 control individuals.

Zafra et al. (2007) analyzed the putative role of a 3′ untranslated region (3′ UTR) polymorphism of the interleukin (IL)12B gene on Chagas disease in 460 Colombian individuals (seronegative: 200; seropositive: 260, with 130 ASY and 130 CCC). They observed a significantly higher frequency of the IL1-2B 3′ UTR CC genotype and of the IL1-2B 3′ UTR C allele in CCC patients. The same authors (Zafra et al., 2008) analyzed the polymorphism of toll-like receptor 2 and 4 genes in 475 Colombian patients (132 ASY, 143 CCC, and 200 seronegative controls). They recorded no frequency differences between chagasic patients and controls.

Ramasawmy et al. (2007) analyzed the polymorphism of the gene coding for lymphotoxin-α in 76 ASY and 169 CCC patients. Homozygosity for the LTA + 80C and LTA + 252G alleles was significantly more frequent in CCC patients than in ASY patients. Haplotype LTA + 80A-252A appeared to have a protective effect against CCC, whereas haplotype LTA + 80C-252G was associated with CCC susceptibility.

Robledo et al. (2007) observed no link between Chagas disease and the variants of the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene in 316 chagasic patients versus 520 healthy controls in Colombia and Peru.

Costa et al. (2009) found that the IL-10 gene polymorphism and IL-10 expression seem to be strongly involved in CCC susceptibility.

Cruz-Robles et al. (2009) surveyed 86 seropositive patients (28 ASY and 58 CCC), 50 seronegative individuals with idiopathic dilated cardiomyopathy (IDC), and 109 control individuals for the distribution of IL-1B and IL-1 receptor antagonist (IL-1RN) variants. Seropositive individuals showed a higher frequency of the CC genotype of the IL-1RN4 polymorphism. CCC patients exhibited an increased frequency of the C allele and of the CC genotype of this polymorphism.

Ramasawmy et al. (2009) analyzed 76 ASY and 169 CCC patients for their variability in the MAL/TIRAP gene, which expresses an adaptor protein in the toll-like receptor pathway. Contrary to Zafra et al. (2008), who found no links between Chagas disease and the genes of the toll-like receptor pathway, these authors observed a protective role against CCC of heterozygosity in the S180L variant of the gene under survey.

Calzada et al. (2009) explored the transforming growth factor beta 1 (TGFβ1) gene polymorphisms in 626 individuals from Colombia and Peru (ASY = 175; CCC = 172; seronegative controls = 279). The frequency of the high TGFβ1

producer genotype 10 C/C was significantly increased in chagasic patients by comparison with seronegative control individuals.

This summarizes the present state of knowledge on human genetic susceptibility to Chagas disease. Although significant progress has been made in the last 15 years, many aspects could be fruitfully explored, including: (i) the possible role of major genomic rearrangements (Check, 2005; Conrad et al., 2010); (ii) the ethnic diversity parameter; and (iii) the linkage and GWAS approaches.

23.5 Vector Genetic Diversity

Chagas disease exhibits a specific epidemiological feature, namely, that the parasite can be transmitted by an impressive range of different vectors. They all pertain to the category of "true bugs" (order Hemiptera, suborder Heteroptera). They are all included in the subfamily Triatominae, family Reduviidae. While other Reduviidae are predators, the Triatominae have specialized in obligatory blood-feeding, including adults of both sexes and larvae. Within the subfamily Triatominae, three main genera of unequal ecogeographic distribution can transmit Chagas disease, namely *Triatoma*, *Rhodnius*, and *Panstrongylus*. Each of these genera includes various species that are able to transmit the disease.

The genetic diversity of the vectors at both the genera and the species levels is therefore considerable.

At the subspecific level, many studies have explored the diversity of many species, both by population genetic markers (see Chapter 15) and by computer-assisted morphometric analysis (see Chapter 16); therefore, the diversity of Chagas disease vectors at the subspecific and population levels is fairly well known.

However, little is known about the differential vectorial capacity of the various triatomine species and of different populations within species. The null hypothesis that all species and all populations are equally able to transmit *T. cruzi* and its various genotypes (see Section 23.6) can be ruled out. It is highly conceivable that refined coevolution phenomena have occurred, meaning that local vectors are better able to transmit local parasite genotypes. This remains to be explored. It is worth noting, however, that a North American vector (*Triatoma protracta*) is fully able to transmit a Latin American strain of *T. cruzi* (Theis et al., 1987).

23.6 Parasite Genetic Diversity

It is interesting to note that, although the scientific community working on Chagas disease is small compared to the numbers working on AIDS, malaria, or tuberculosis, this pathogen has long been among the pioneer species explored by advanced approaches such as molecular typing and **population genetics**. Therefore, this parasite is probably the pathogen whose evolutionary biology is the best known, together with *Escherichia coli*. It can therefore be suggested as a paradigmatic biological model, as has been done with *E. coli*, *Drosophila melanogaster*, *Mus musculus*, and *Caenorhabditis elegans* (Tibayrenc, 2009).

Pioneering molecular studies on *T. cruzi* explored **isoenzyme** variability as early as the beginning of the 1970s (Toyé, 1974). Although a now out-of-fashion technique, **multilocus enzyme electrophoresis** has clearly discriminated three principal variants or zymodemes within *T. cruzi* (Miles et al., 1978). It is interesting to note that this observation remains current, since these three zymodemes continue to be recorded today in *T. cruzi* natural populations, although their denomination and evolutionary status has changed substantially. This permanency of **multilocus genotypes** over space and time is one of the strongest arguments in favor of predominant clonal evolution (see later).

The interpretation of isoenzyme diversity in terms of population genetics and evolutionary biology has made it possible to clarify the evolutionary status of the zymodemes. The model of predominant clonal evolution has been proposed for *T. cruzi* (Tibayrenc et al., 1986) as well as for other parasitic protozoa (Tibayrenc et al., 1990). The evidence was mainly based on the observation of a considerable linkage disequilibrium (nonrandom association of genotypes occurring at different loci). Linkage disequilibrium is the very manifestation of very limited or absent genetic recombination. The model stipulates that offspring multilocus genotypes are virtually identical to the parental genotypes and are stable in space and time, whatever the precise cytological mechanism of propagation. The model therefore includes not only mitotic propagation but also various forms of parthenogenesis, extreme homogamy, and self-fertilization in haploid organisms (Tibayrenc et al., 1990). Extreme inbreeding is not an alternative model to clonal evolution (Rougeron et al., 2009), but rather a particular case of it.

The main relevance of the model concerns molecular epidemiology (tracing multilocus genotypes [strains] with molecular tools for epidemiological follow-up). If predominant clonal evolution inhibits recombination, as stated earlier, the multilocus genotypes are stable in space and time, even at an evolutionary scale, and therefore constitute convenient targets for molecular epidemiology.

Since its inception, the clonal model has stated that it was compatible with occasional bouts of genetic recombination. Recombination has long been suspected in natural populations of *T. cruzi* (Machado and Ayala, 2001) and has been experimentally evidenced (Gaunt et al., 2003). However, it is clear that such hybridization events interfere only at an evolutionary scale. The stability of *T. cruzi* multilocus genotypes in the long run, with its extreme manifestation of strong parity between **phylogenetic** trees designed from different genetic markers (Tibayrenc et al., 1993) is incompatible with frequent genetic recombination.

It has been suggested that *T. cruzi* genotypes are distributed into six different clusters (Barnabé et al., 2000; Brisse et al., 2000), which cannot be equated with real **clades** because some of them clearly originate from former hybridization events (Brisse et al., 2003; Sturm and Campbell, 2010), further stabilized by clonal propagation. The term "discrete typing unit" (DTU; Tibayrenc, 1998a) has been coined to designate sets of stocks that are genetically closer to each other than to any other stock and are identifiable by common molecular, genetic, biochemical, or immunological markers called tags. The six *T. cruzi* clusters match this definition.

Their validity has been confirmed at a recent meeting of Chagas disease experts (Zingales et al., 2009).

From the points of view of molecular epidemiology and integrated genetic epidemiology, the population structure of *T. cruzi* summarized earlier can be illustrated by two key words: stability and discreteness. *T. cruzi* natural clones, and the DTUs into which they are distributed, are genetic entities that are both stable in space and time (up to the evolutionary scale) and strictly separated from each other, with rare occasional bouts of genetic exchange.

23.7 Concluding Remarks

The data described herein was not intended to be a comprehensive review of our present knowledge on the genetic diversity of Chagas disease hosts, vectors, and parasites. Instead, the goal was to briefly highlight why these data make Chagas disease a good model for the integrated genetic epidemiology of infectious diseases, as already proposed long ago (Tibayrenc, 1998b).

The key word here again is discreteness: discreteness of the clinical phenotypes of Chagas disease in humans, discreteness of *T. cruzi* clonal genotypes and DTUs, discreteness of the many different species that are hosts (mammals) and vectors (triatomine bugs) of Chagas disease. All these discrete entities can be used as units of analysis, keys on the keyboard to be played in many different situations that can be analyzed, both in surveying natural Chagas cycles and in designing experimental evolution protocols.

There are several possible examples.

When natural cycles are considered, possible protocols could be to compare *T. cruzi* genotypes isolated from (i) cardiac versus digestive versus ASY patients, (ii) different mammal species, and (iii) different triatomine bug species.

Experimental evolution protocols are easy because: (i) *T. cruzi* is easy to culture; (ii) many triatomine bug species are easy to raise; and (iii) several experimental animal models are available, and one can compare, for example, different breeds of mice, whose genetic distinctness results in differing susceptibility to Chagas disease.

All this makes the integrated genetic epidemiology of Chagas disease an extremely promising field of research that has until now been underexplored. It could constitute a paradigmatic example to develop similar approaches in other infectious models.

Glossary

Clade evolutionary lineage defined by cladistic analysis. A clade is monophyletic (it has only a single ancestor) and is genetically isolated (which means that it evolves independently) from other clades.

Isoenzymes, multilocus enzyme electrophoresis protein extracts of given biological samples are separated by electrophoresis. The gel is then processed with a biochemical reaction involving the specific substrate of a given enzyme. This enzyme's zone of activity is then specifically stained on the gel. From one sample to another, migration differences can appear for this same enzyme. These different electrophoretic forms of the same enzyme are referred to as isoenzymes or isozymes. These differences reflect sequence differences in the genes coding for the involved enzymes.

Multilocus genotype the combined genotype of a given strain or a given individual established with several genetic loci.

Phenotype all observable properties of a given individual or a given population apart from the genotype. The phenotype is not limited to morphological characteristics and can include, for example, physiological or biochemical parameters. The pathogenicity of a microorganism is a phenotypic property, as are the different clinical forms of a given disease. The phenotype is produced by the interaction between genotype and the environment.

Phylogeny, phylogenetic evolutionary relationships between taxa, species, organisms, genes, or molecules.

Population genetics analysis of allele and genotype frequency distribution and modifications under the influence of natural selection, mutation, genetic drift, and gene flow.

References

Abel, L., Sanchez, F.O., Oberti, J., Thuc, N.V., Hoa, L.V., Lap, V.D., et al., 1998. Susceptibility to leprosy is linked to the human NRAMP1 gene. J. Infect. Dis. 177, 133–145.

Alric, L., Fort, M., Izopet, J., Vinel, J.P., Duffaut, M., Abbal, M., 1999. Association between genes of the major histocompatibility complex class II and the outcome of hepatitis C virus infection. J. Infect. Dis. 179, 1309–1310.

Apt, W., 1988. HLA Antigens in cardiomyopathic Chilean chagasics. Am. J. Hum. Genet. 43, 770–773.

Barbossa, C.A.A., Morton, N.E., Pao, D.C., Krieger, H., 1981. Biological and cultural determinants of immunoglobulin levels in a Brazilian population with Chagas' disease. Hum. Genet. 59, 161–163.

Barnabé, C., Brisse, S., Tibayrenc, M., 2000. Population structure and genetic typing of *Trypanosoma cruzi*, the agent of Chagas' disease: a multilocus enzyme electrophoresis approach. Parasitology 150, 513–526.

Bellamy, R., Beyers, N., McAdam, K.P., Ruwende, C., Gie, R., Samaai, P., et al., 2000. Genetic susceptibility to tuberculosis in Africans: a genome-wide scan. Proc. Natl. Acad. Sci. U.S.A. 97, 8005–8009.

Borrs, S.G., Racca, L., Cotorruelo, C., Biondi, C., Beloscar, J., Racca, A., 2009. Distribution of HLA-DRB1 alleles in Argentinean patients with chagas' disease cardiomyopathy. Immunol. Invest. 38, 268–275.

Brenière, S.F., Poch, O., Selaes, H., Tibayrenc, M., Lemesre, J.L., Antezana, G., et al., 1984. Specific humoral depression in chronic patients infected by *Trypanosoma cruzi*. Rev. Inst. Med. Trop. Sao Paulo 26, 254–258.

Brisse, S., Barnabé, C., Tibayrenc, M., 2000. Identification of six *Trypanosoma cruzi* phylogenetic lineages by random amplified polymorphic DNA and multilocus enzyme electrophoresis. *Int. J. Parasitol*. 30, 35−44.

Brisse, S., Henriksson, J., Barnabé, C., Douzery, E.J.P., Berkvens, D., Serrano, M., et al., 2003. Evidence for genetic exchange and hybridization in *Trypanosoma cruzi* based on nucleotide sequences and molecular karyotype. Infect. Genet. Evol. 2, 173−183.

Bucheton, B., Kheir, M.M., El-Safi, S.H., Hammad, A., Mergani, A., Mary, C., et al., 2002. The interplay between environmental and host factors during an outbreak of visceral leishmaniasis in eastern Sudan. Microbes Infect. 4, 1449−1457.

Calzada, J.E., Nieto, A., Beraún, Y., Martín, J., 2001a. Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. Tissue Antigens 58, 154−158.

Calzada, J.E., Nieto, A., López-Nevot, M.A., Martín, J., 2001b. Lack of association between NRAMP1 gene polymorphisms and *Trypanosoma cruzi* infection. Tissue Antigens 57, 353−357.

Calzada, J.E., Beraún, Y., González, C.I., Martín, J., 2009. Transforming growth factor beta 1 (TGFβ1) gene polymorphisms and Chagas disease susceptibility in Peruvian and Colombian patients. Cytokine 45, 149−153.

Campelo, V., Dantas, R.O., Simões, R.T., Mendes-Junior, C.T., Sousa, S.M.B., Simões, A.L., et al., 2007. TNF microsatellite alleles in Brazilian chagasic patients. Dig. Dis. Sci. 52, 3334−3339.

Check, E., 2005. Patchwork people. Nature 437, 1084−1086.

Conrad, D.F., Pinto, D., Redon, R., Feuk, L., Gokcumen, O., Zhang, Y., et al., 2010. Origins and functional impact of copy number variation in the human genome. Nature 464, 704−712.

Costa, G.C., Rocha, M.O.D.C., Moreira, P.R., Menezes, C.A.S., Silva, M.R., Gollob, K.J., et al., 2009. Functional IL-10 gene polymorphism is associated with Chagas disease cardiomyopathy. J. Infect. Dis. 199, 451−454.

Cruz-Robles, D., Reyes, P.A., Monteón-Padilla, V.M., Ortiz-Muñiz, A.R., Vargas-Alarcón, G., 2004. MHC class I and class II genes in Mexican patients with Chagas disease. Hum. Immunol. 65, 60−65.

Cruz-Robles, D., Chvez-Gonzlez, J.P., Cavazos-Quero, M.M., Prez-Mndez, O., Reyes, P.A., Vargas-Alarcn, G., 2009. Association between IL-1B and IL-1RN gene polymorphisms and chagas' disease development susceptibility. Immunol. Invest. 38, 231−239.

Dean, M., Carrington, M., Winckler, C., Huttley, G.A., Smith, M.W., Allikmets, R., et al., 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Science 273, 1856−1862.

Dessein, A.J., Marquet, S., Henri, S., El Wali, N.E., Hillaire, D., Rodrigues, V., et al., 1999. Infection and disease in human schistosomiasis mansoni are under distinct major gene control. Microbes Infect. 1, 561−567.

Drigo, S.A., Cunha-Neto, E., Ianni, B., Cardoso, M.R.A., Braga, P.E., Faé, K.C., et al., 2006. TNF gene polymorphisms are associated with reduced survival in severe Chagas' disease cardiomyopathy patients. Microbes Infect. 8, 598−603.

Drigo, S.A., Cunha-Neto, E., Ianni, B., Mady, C., Faé, K.C., Buck, P., et al., 2007. Lack of association of tumor necrosis factor-α polymorphisms with Chagas disease in Brazilian patients. Immunol. Lett. 108, 109−111.

Faé, K.C., Drigo, S.A., Cuha-Neto, E., Ianni, B., Mady, C., Kalil, J., et al., 2000. HLA and beta-myosin heavy chain do not influence susceptibility to Chagas disease cardiomyopathy. Microbes Infect. 2, 745−751.

Fernandes-Mestre, M.T., Layrisse, Z., Montagnani, S., Acquatella, H., Catalioti, F., Matos, M., et al., 1998. Influence of the HLA class II polymorphism in chronic Chagas disease. Parasite Immunol. 20, 197–203.

Flórez, O., Zafra, G., Morillo, C., Martín, J., González, C.I., 2006. Interleukin-1 gene cluster polymorphism in Chagas disease in a Colombian case–control study. Hum. Immunol. 67, 741–748.

Garcia, A., Marquet., S., Bucheton, B., Hillaire, D., Cot, M., Fievet, N., et al., 1998. Linkage analysis of blood *Plasmodium falciparum* levels: interest of the 5q31-q33 chromosome region. Am. J. Trop. Med. Hyg. 58, 705–709.

Gaunt, M.W., Yeo, M., Frame, I.A., Tothard, J.R., Carrasco, H.J., Taylor, M.C., et al., 2003. Mechanism of genetic exchange in American trypanosomes. Nature 421, 936–939.

Layrisse, Z., Fernandez, M.T., Montagnani, S., Matos, M., Balbas, O., Herrera, F., et al., 2000. HLA-C*03 is a risk factor for cardiomyopathy in Chagas disease. Hum. Immunol. 61, 925–929.

Machado, C.A., Ayala, F.J., 2001. Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of *Trypanosoma cruzi*. Proc. Nat. Acad. Sci. U.S.A. 98, 7396–7401.

Messias-Reason, I.J., Urbanetz, L., Pereira Da Cunha, C., 2003. Complement C3 F and BFS allotypes are risk factors for Chagas disease cardiomyopathy. Tissue Antigens 62, 308–312.

Miles, M.A., Souza, A., Povoa, M., Shaw, J.J., Lainson, R., Toyé, P.J., 1978. Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochtonous patients with Chagas' disease in Amazonian Brazil. Nature 272, 819–821.

Moreno, M., Silva, E.L., Ramírez, L.E., Palacio, L.G., Rivera, D., Arcos-Burgos, M., 2004. Chagas' disease susceptibility/resistance: linkage disequilibrium analysis suggest epistasis between major histocompatibility complex and interleukin-10. Tissue Antigens 64, 18–24.

Morini, J.C., Berra, H., Davila, H.O., Pividori, J.F., Bottasso, O.A., 1994. Electrocardiographic alteration among first degree relatives with serological evidence of *Trypanosoma cruzi* infection. A sibship study. Mem. Inst. Oswaldo Cruz 89, 371–375.

Nieto, A., Beraún, Y., Callado, M.D., Caballero, A., Alonso, A., González, A., et al., 2000. HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection. Tissue Antigens 55, 195–198.

Pennisi, E., 2007. Human genetic variation. Science 318, 1842–1843.

Ramasawmy, R., Cunha-Neto, E., Faé, K.C., Müller, N.G., Cavalcanti, V.L., Drigo, S.A., et al., 2006a. BAT1, a putative anti-inflammatory gene, is associated with chronic chagas cardiomyopathy. J. Infect. Dis. 193, 1394–1399.

Ramasawmy, R., Cunha-Neto, E., Faé, K.C., Martello, F.G., Müller, N.G., Cavalcanti, V.L., et al., 2006b. The monocyte chemoattractant protein-1 gene polymorphism is associated with cardiomyopathy in human Chagas disease. Clin. Infect. Dis. 43, 305–311.

Ramasawmy, R., Fae, K.C., Cunha-Neto, E., Müller, N.G., Cavalcanti, V.L., Ferreira, R.C., et al., 2007. Polymorphisms in the gene for lymphotoxin-α predispose to chronic chagas cardiomyopathy. J. Infect. Dis. 196, 1836–1843.

Ramasawmy, R., Faé, K.C., Cunha-Neto, E., Borba, S.C.P., Ianni, B., Mady, C., et al., 2008. Variants in the promoter region of IKBL/NFKBIL1 gene may mark susceptibility to the development of chronic Chagas' cardiomyopathy among *Trypanosoma cruzi*-infected individuals. Mol. Immunol. 45, 283–288.

Ramasawmy, R., Cunha-Neto, E., Faé, K.C., Borba, S.C.P., Teixeira, P.C., Ferreira, S.C.P., et al., 2009. Heterozygosity for the S180L variant of MAL/TIRAP, a gene expressing

an adaptor protein in the toll-like receptor pathway, is associated with lower risk of developing chronic chagas cardiomyopathy. J. Infect. Dis. 199, 1838–1845.

Robledo, G., González, C.I., Morillo, C., Martín, J., González, A., 2007. Association study of PTPN22 C1858T polymorphism in *Trypanosoma cruzi* infection. Tissue Antigens 69, 261–264.

Rodrigues Coura, J., Albajar Viñas, P., 2010. Chagas disease: a new world challenge. Nature 465, S6–S7.

Rougeron, V., De Meeûs, T., Hide, M., Waleckx, E., Bermudez, H., Arevalo, J., et al., 2009. Extreme inbreeding in *Leishmania braziliensis*. Proc. Nat. Acad. Sci. U.S.A. 25, 10224–10229.

Sturm, N., Campbell, D.A., 2010. Alternative lifestyles: the population structure of *Trypanosoma cruzi*. Acta Trop. 115, 35–43.

Theis, J.H., Tibayrenc, M., Mason, D.T., Ault, S.K., 1987. Exotic stock of *Trypanosoma cruzi* (*Schizotrypanum*) capable of development in and transmission by *Triatoma protracta protracta* from California. Public health implications. *Am. J. Trop. Med. Hyg*. 36, 523–528.

Tibayrenc, M., 1998a. Genetic epidemiology of parasitic protozoa and other infectious agents: the need for an integrated approach. *Int. J. Parasitol*. 28, 85–104.

Tibayrenc, M., 1998b. Integrated genetic epidemiology of infectious diseases: the Chagas model. Mem. Inst. Oswaldo Cruz 93, 577–580.

Tibayrenc, M., 2007. Human genetic diversity and epidemiology of parasitic and other transmissible diseases. Adv. Parasitol. 64, 378–428.

Tibayrenc, M., 2009. Modeling the transmission of *Trypanosoma cruzi*: the need for an integrated genetic epidemiological and population genomics approach. In: Michael, E. (Ed.), Infectious Disease Transmission Modeling and Management of Parasite Control. Landes Bioscience/Eurekah, Austin, TX.

Tibayrenc, M., Ward, P., Moya, A., Ayala, F.J., 1986. Natural populations of *Trypanosoma cruzi*, the agent of Chagas' disease, have a complex multiclonal structure. Proc. Nat. Acad. Sci. U.S.A. 83, 115–119.

Tibayrenc, M., Kjellberg, F., Ayala, F.J., 1990. A clonal theory of parasitic protozoa: the population structure of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas* and *Trypanosoma*, and its medical and taxonomical consequences. Proc. Nat. Acad. Sci. U.S.A. 87, 2414–2418.

Tibayrenc, M., Neubauer, K., Barnabé, C., Guerrini, F., Sarkeski, D., Ayala, F.J., 1993. Genetic characterization of six parasitic protozoa: parity of random-primer DNA typing and multilocus isoenzyme electrophoresis. Proc. Natl. Acad. Sci. U.S.A. 90, 1335–1339.

Toyé, P.J., 1974. Isoenzymic differences between culture forms of *Trypanosoma rangeli*, *T. cruzi*, and *T. lewisi*. *Trans. R. Soc. Trop. Med. Hyg*. 68, 266.

Visentainer, J.E., Moliterno, R.A., Sell, A.M., Petzel-Erler, M.L., 2002. Association of HLA-DR2 with chronic chagasic cardiopathy in a population at Paraná Northeast region, Brazil. Acta Sci. Biol. Health Sci. 24, 727–730.

Williams-Blangero, S., VandeBerg, J.L., Blangero, J., Teixera, A.R.L., 1997. Genetic epidemiology of seropositivity for *T. cruzi* infection in rural Goiás, Brazil. Am. J. Trop. Med. Hyg. 57, 538–543.

Zafra, G., Morillo, C., Martín, J., González, A., González, C.I., 2007. Polymorphism in the 3′ UTR of the IL12B gene is associated with Chagas' disease cardiomyopathy. Microbes Infect. 9, 1049–1052.

Zafra, G., Flórez, O., Morillo, C.A., Echeverría, L.E., Martín, J., González, C.I., 2008. Polymorphisms of toll-like receptor 2 and 4 genes in Chagas disease. Mem. Inst. Oswaldo Cruz 103, 27−30.

Zicker, F., Slith, P.G., Netto, J.C.A., Oliveira, R.M., Zicke, E.M.S., 1990. Physical activity, opportunity for reinfection, and sibling history of heart diseases as risk factors for Chagas' cardiopathy. Am. J. Trop. Med. Hyg. 43, 498−505.

Zingales, B., Andrade, S.G., Briones, M.R.S., Campbell, D.A., Chiari, E., Fernandes, O., et al., 2009. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem. Inst. Oswaldo Cruz 104, 1051−1054.

Tibayrenc Michel, Telleria Jenny, Diosque P., Dib J.C., Barnabé Christian.

Integrated genetic epidemiology of Chagas disease.

In : Tibayrenc Michel (ed.). Genetics and evolution of infectious diseases. Amsterdam : Elsevier, 2011, p. 637-688.

(Elsevier Insights Series). ISBN 978-0-12-384890-1