Published in final edited form as:

Adv Parasitol. 2011 ; 75: 147–167. doi:10.1016/B978-0-12-385863-4.00007-1.

Genetic Epidemiology of Chagas Disease

Sarah Williams-Blangero, Ph.D.¹, John L. VandeBerg, Ph.D.^{1,2}, John Blangero, Ph.D.¹, and Rodrigo Corrêa-Oliveira, Ph.D.^{3,4}

¹Texas Biomedical Research Institute, Department of Genetics, San Antonio, Texas, 78227, USA;

²Southwest National Primate Research Center, San Antonio, Texas, 78227, U.S.A.;

³Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil;

⁴Instituto Nacional de Ciencia e Tecnologia em Doenças Tropicais - INCTDT.

Abstract

Genetic epidemiological approaches hold great promise for improving understanding of the determinants of susceptibility to infection with *Trypanosoma cruzi* and the causes of differential disease outcome in *T. cruzi* infected individuals. To date, a variety of approaches have been used to understand the role of genetic factors in Chagas disease. Quantitative genetic techniques have been used to estimate heritabilities for seropositivity for *T. cruzi* infection and traits that are associated with disease progression in chronic *T. cruzi* infection. These studies have demonstrated that a significant proportion of the variation in seropositivity and a number of traits related to Chagas disease progression is attributable to genetic factors. Candidate gene studies have provided intriguing evidence for the roles of numerous individual genes in determining cardiac outcomes in chronically infected individuals. Recent results from a long-term study of Chagas disease in a rural area of Brazil has documented that over 60% of the variation in seropositivity status is attributable to genetic factors in that population. Additionally, there are significant genetic effects on a number of electrocardiographic measures and other Chagas disease related traits. The application of genome wide approaches will yield new evidence for the roles of specific genes in Chagas disease.

1. INTRODUCTION

Over 100 years after the parasitic cause of Chagas disease, or American trypanosomiasis, was elucidated, *Trypanosoma cruzi* infection and chronic Chagas disease persist as major public health problems throughout South and Central America (WHO, 2010). Chagas disease is still one of the leading causes of cardiac disease in Latin America. It is estimated that 8 to 10 million people are infected with *Trypanosoma cruzi*, and that tens of millions more are at risk for infection (Dutra et al., 2005; WHO, 2010; Rassi et al., 2010; Sanchez-Sancho et al., 2010).

Despite the success of vector control programs in some geographic regions, active transmission occurs in many areas of Latin America (e.g., Gurtler et al., 1992; Rizzo et al., 2003; Grijalva et al., 2003; Coll-Cardenas et al., 2004; Pinto et al., 2004; Borges et al., 2006). The disease is emerging in new geographic areas both in Latin America and elsewhere in the world (e.g., Briceño-León, 2007; Aguilar et al., 2007; Calzada et al., 2010; Norman et al., 2010). The disease is also increasing in importance as a public health concern

in the U.S. and is now a reportable disease in two states (Leiby et al., 2002; McCarthy, 2003; Busch et al., 2003; Beard et al., 2003; Bern et al., 2009; Sarkar et al., 2010).

Chagas disease generally occurs in two phases. First, there is an acute phase lasting 2–3 months following infection with *T. cruzi* (Kirchhoff, 1999). Many individuals are asymptomatic throughout the acute phase of the illness, although between 5% and 10% of individuals experience severe, sometimes fatal disease during this period (Kirchhoff, 1999). About 40% of those infected who survive the acute phase remain seropositive but never experience disease progression. In the remaining 60% of cases, the disease enters a quiescent phase lasting as long as 30 years. Out of this population it is estimated that 2% will enter the chronic phase of the disease each year. The chronic phase is characterized by progressive cardiomyopathy, or by a digestive form of the disease associated with mega-colon and/or mega-esophagus.

The cardiac form of chronic Chagas disease is evident in data generated from electrocardiograms. The characteristics associated with Chagas disease include bradyarrhythmias, premature ventricular contractions, atrio-ventricular blocks, and right bundle branch blocks (Maguire et al., 1987; Kirchhoff, 1999; Prata, 2001; Rangel-Flores et al., 2001; Yacoub et al., 2003; Sosa-Jurado et al., 2003; Jorge et al., 2003; Goldbaum et al., 2004; Williams-Blangero et al., 2007; Biolo et al., 2010). Some ECG characteristics such as an elongated QT interval have been suggested to predict mortality in Chagasic patients (Salles et al., 2003, 2004).

There are no drugs that can be taken to prevent infection with the *T. cruzi* parasite (Urbina and Docampo, 2003; Garg and Bhatia, 2005). Two drugs have been used for the treatment of acute *T. cruzi* infection, nifurtimox and benznidazole. Although these drugs are effective in decreasing early parasitemia during the acute phase of disease, the evidence for their efficacy during the chronic phase of Chagas disease is inconclusive (Rodriguez Coura, 2002; Castro et al., 2006; Tanowitz et al., 2009; Lescure et al., 2010). Both drugs are carcinogenic in a variety of animal models (Garcia Zapata and Marsden, 1986; Teixeira et al., 1990a; 1990b; Castro et al., 2006), and they have a broad range of side effects which make them difficult to tolerate for the 1 to 3 months of treatment routinely administered (Castro et al., 2006; Tanowitz et al., 2009; Lescure et al., 2010). As a result, there is no consensus on their use in treatment of the chronic phase of the disease in adults (Tanowitz et al., 2009).

The lack of generally accepted pharmacological intervention for chronic Chagas disease highlights the value of genetic studies for this disease. Modern genetic approaches can facilitate the detection of potential novel drug targets based on genetic analysis of differential disease progression in *T. cruzi* infected individuals. The identification of genes influencing Chagas disease can suggest novel biological pathways to be targeted in drug development efforts (Ohlstein et al., 2000; Schadt et al., 2003). In addition, the novel pathways identified by genetic analysis may be treatable with existing pharmaceutical drugs. Application of existing compounds to novel pathways in the disease could lead to relatively rapid availability of new treatments for Chagas disease.

2. Genetic Epidemiological Approaches to Chagas Disease

Genetic epidemiological approaches can be informative for improving our understanding of the determinants of differential susceptibility to *T. cruzi* infection and differential disease outcome in individuals who are chronically infected with *T. cruzi*. Because uninfected individuals are found in all populations which experience high rates of Chagas disease, the question of whether or not there is differential susceptibility to *T. cruzi* infection naturally arises. There is familial clustering of seronegativity for *T. cruzi* infection, and genetic epidemiological studies may be designed to assess whether or not there is a genetic component to differential susceptibility to infection, assuming universal exposure to risk of infection in the population.

Among seropositive individuals, there is significant variation in disease progression. Some individuals remain asymptomatic throughout their lives, while others experience varying degrees of cardiac involvement. The question of whether or not there is a genetic component to disease progression in infected individuals is also an interesting one which can be addressed using genetic epidemiological approaches. Disease progression can be measured in a multitude of ways. Immunological correlates of Chagas disease and electrocardiogram variables provide measures of progression of cardiac Chagas disease that can be subjected to genetic analysis using a variety of approaches.

Depending upon the type of genetic epidemiological methods used, one can determine the percent of variation in the Chagas disease-related trait attributable to genetic factors, the chromosomal location of genetic effects on the traits, the specific genes involved in determination of the traits, and the individual genetic variants responsible for observed patterns of variation in disease related traits. The knowledge of specific genes involved in disease-related traits potentially may facilitate the development of powerful tools for improving public health. Genetic information may identify novel pathways to target in drug development efforts. It may also identify pathways that may be modulated by existing drugs already on the market for other purposes. Finally, genetic information may provide a means for developing predictive tests to assess likelihood of disease progression.

There is great value to being able to reliably predict disease progression in seropositive individuals. As noted above, the available treatments for Chagas disease are generally toxic and poorly tolerated. Development of a reliable prognostic test capable of identifying those individuals who will progress to disease will allow targeting of available treatments to the individuals most likely to develop disease.

Besides the potential role of host genetic factors, genetic variation within the pathogen may also play a role in susceptibility. Substantial effort has been devoted towards documenting the genetic variation among *Trypanosoma cruzi* organisms (reviewed by Macedo et al., 2004; Campbell et al., 2004; Manuel-Caetano and Silva, 2007; Miles et al., 2009). Genetic variation in the parasitic organism *Trypanosoma cruzi* has been implicated in differential disease outcome, but the importance of that impact remains to be fully elucidated (Sturm et al., 2003; Buscaglia and Noia, 2003; Macedo et al., 2004). Severe cardiac outcomes have been observed in infections with both Type I and Type II lineages of *Trypanosoma cruzi*

(e.g., Añez et al., 2004). Differences in disease epidemiology between major geographic areas, such as the lack of the digestive form of Chagas disease in Venezuela, have been hypothesized to be due to variation in the parasite. However, it is acknowledged that environmental, nutritional, and host immunological factors may also account for this variation (Macedo et al., 2004). While parasite genetic factors are likely to play a role in determining differential outcome in Chagas disease, the precise nature of this role remains to be determined. Much work remains to be done before the role of parasite genetics in Chagas disease is fully understood (Sturm et al., 2003).

Tibayrenc (1998, 2007, 2010) has emphasized the importance of considering both genetic variation in parasites and hosts in the study of Chagas disease. He has recommended developing an integrated genetic epidemiological model for infectious disease that incorporates consideration of genetic variation present in the parasite, the vector, and the host when working to understand the population biology of a disease like Chagas disease.

2.1 Epidemiological observations of familial clustering

Before an expensive search for individual genes influencing any disease is launched, there must be documented evidence of the involvement of genes in the disease. Familial clustering of a disease is one of the most basic pieces of evidence that genetic factors are involved in the determination of a disease. The clustering of Chagas disease in familial households has long been reported (e.g., Mott et al., 1976; Zicker et al., 1990; Tibayrenc, 1998, 1999). Most frequently, this clustering is attributed to environmental factors that correlate with household and that are thought to influence exposure to the vector. However, in one examination of familial clustering of seropositivity for *T. cruzi* infection and cardiac outcomes of Chagas disease in a population from Brazil, the possibility of genetic influences was explicitly considered (Silva-Grecco et al., 2010). In this study of 41 families ranging in size from 5 to 80 individuals, there was significant evidence for familial factors influencing both seropositivity and cardiac outcome traits, although no explicit statistical evidence for the role of a major gene with large effect in determining variation was found (Silva-Grecco et al., 2010).

2.2 Analyses of the heritability of Chagas disease related traits

The heritability of a disease-related trait provides an overall estimate of the importance of genetic factors in determining that trait. For example, a heritability of 0.7 for a given trait indicates that approximately 70% of the variation in that trait is attributable to genetic factors within the sample examined.

There have been few quantitative genetic assessments of Chagas disease or Chagas disease related traits. One of the first such studies assessed the biological and cultural correlates of immunoglobulin levels in an endemic Brazil population (Barbosa et al., 1981). This study utilized a path analysis approach to estimate heritabilities of approximately 30% for both IgA and IgG, and of approximately 10% for IgM (Barbosa et al., 1981). The pedigrees, which included a total of 390 individuals, for this study were built around 40 year old patients who were in the chronic phase of Chagas disease.

For our own quantitative genetic studies of Chagas disease and related phenotypes, we generated pedigrees for families in the Posse region of Goiás through house to house surveys following the approach outlined in Williams-Blangero and Blangero (2006). Our study of the heritability of seropositivity for *T. cruzi* infection included a total of 525 individuals who belonged to a total of 146 pedigrees ranging in size from 2 to 103 individuals. In addition, we included 179 independent individuals to improve parameter estimation (Williams-Blangero et al., 1997). Analyzing the dichotomous trait of seropositivity on an assumed underlying continuous distribution of liability, we determined the heritability of seropositivity for *T. cruzi* infection to be 0.56 indicating that over half of the variation in seropositivity for *T. cruzi* infection is attributable to genetic factors. These results strongly suggest a genetic component to susceptibility to infection with *T. cruzi*.

2.3 Candidate gene studies of Chagas disease

Given that family studies support the likelihood of genetic factors playing a role in Chagas disease susceptibility and response, one approach to causal gene discovery is to focus directly on known candidate genes on the basis of our cumulative biological knowledge regarding the underlying pathways involved in the disease. Once a candidate gene is chosen, it can be examined closely for potential variation that influences quantitative variation in the focal risk factors. The central difficulty with the classical candidate gene approach is the low prior probability of picking a functional gene given our relatively incomplete knowledge about the biological processes underlying disease. Additionally, the classical candidate gene approach is limited to incremental progress since much has to be already known about the gene to nominate it as a candidate. Novel discoveries are thus minimized using the classical candidate gene approach.

Ramasawmy and colleagues of the Heart Institute at the University of São Paulo School of Medicine in Brazil have conducted a number of candidate gene studies assessing the genetic effects of candidate genes on risk for development of chronic Chagas cardiomyopathy in individuals seropositive for *T. cruzi* infection (Ramasawmy et al., 2006, 2007, 2008, 2009). By assessing variation in specific genes in infected individuals with Chagas-related cardiomyopathy and asymptomatic individuals who are seropositive for *T. cruzi* infection, Ramasawmy and colleagues have provided intriguing although relatively statistically weak evidence of genes that may be involved in disease progression in infected individuals. Variation in the monocyte chemoattractant protein-1 gene (CCL2/MCP-1) is associated with development of cardiomyopathy in individuals with *T. cruzi* infection (Ramasawmy et al., 2006). Polymorphisms in the gene for lymphotoxin- α which is a proinflammatory cytokine have been demonstrated to be weakly ($p=0.035$) associated with risk for cardiomyopathy in *T. cruzi* positive individuals (Ramasawmy et al., 2007). Similarly, variation in the inhibitory KappaB-like gene (IKBL) or a gene very close to it has been implicated in differential susceptibility to cardiomyopathy in individuals with chronic Chagas disease (Ramasawmy et al., 2008). A further study demonstrated an association ($p=0.0084$) between heterozygosity for a variant of MAL/TIRAP gene and lowered risk of developing cardiomyopathy given infection with *T. cruzi* (Ramasawmy et al., 2009).

The associations between genes encoding for several interleukins (IL1A, IL1B, and IL1RN) in the interleukin-1 gene cluster and disease progression in Chagas disease were examined using a case control design in symptomatic and asymptomatic seropositive Colombian patients (Flórez et al., 2006). They found haplotype differences between affected and unaffected seropositive individuals that suggest that variation in the IL-1 gene cluster may influence differential susceptibility to the cardiac form of chronic Chagas disease. A similar study conducted in Mexico demonstrated an association between an IL1RN polymorphism and development of cardiac disease in individuals seropositive for *T. cruzi* infection (Cruz-Robles et al., 2009).

The inflammatory cytokine Tumor Necrosis Factor Alpha (TNF- α) is known to be elevated in individuals with the cardiac form of chronic Chagas disease. In studies of a Brazilian population, Drigo and colleagues determined that a specific TNF genotype was associated with risk of death in Chagas patients (Drigo et al., 2006), but did not find *TNFA* polymorphisms to be associated with severity of cardiac disease in another study (Drigo et al., 2007).

The importance of variation in the interferon-gamma gene (*IFNG*) for Chagas disease was examined in a Colombian population (Torres et al., 2010). While they found no association between variation in *IFNG* and differential disease progression in seropositive cases compared to controls, Torres et al. (2010) did find an association between seropositivity for *T. cruzi* infection and *IFNG*, suggesting that variation in the gene may influence differential susceptibility to infection with *T. cruzi*.

Classical markers in the Human Leukocyte Antigen (HLA) system are frequently the focus of candidate gene studies in infectious disease (see review by Blackwell et al., 2009). Many studies have found documented associations between HLA haplotypes and *T. cruzi* infection or development of Chagas disease in infected individuals (Llop et al., 1991; Fernandez-Mestre et al., 1998; Layrisse et al., 2000; Nieto et al., 2000; Colorado et al., 2000; Cruz-Robles et al., 2004; Moreno et al., 2004; García Borrás et al., 2009).

Despite the intriguing nature of these studies of candidate gene associations with various aspects of Chagas disease, most of these studies are based on relatively small samples including at most several hundred individuals. Few of these studies have rigorously controlled for the number of associations tested, thus increasing the chance of observing false positive associations. Additionally, the results may be confounded by differential admixture in the study participants. Many populations in Latin America have high rates of admixture involving European, African, and Amerindian ancestry. It is critical to control for the underlying population stratification in association study designs, including candidate gene assessments. False positive associations generated by simple population differences that are mirrored in neutral genetic variation are a likely outcome of candidate gene studies that fail to appropriately account for underlying population stratification.

2.4 Genome-wide assessments of Chagas disease-related traits

In the post-genomic era, our approach to understanding the genetic architecture of a complex phenotype has generally followed a specific route. First, an underlying QTL is localized by a

genomic scan to a potentially larger chromosomal region. Until the past five years, this localization was usually accomplished by linkage analysis using data on the co-segregation of phenotypes and genetic markers in families. Such linkage mapping relies on information provided by the identity-by-descent status of chromosomal regions evaluated within families. It is able to potentially pick up the signals derived from both common and rare functional genetic variation. Linkage mapping will generally identify a relatively large chromosomal region (10–15 megabases) as having the likelihood of harboring a disease-related QTL.

The second main approach to causal gene localization involves the utilization of an association mapping paradigm. Genome scanning using an association approach is dependent upon local linkage disequilibrium and is capable of finer resolution (generally pinpointing a chromosomal region of approximately 500kb) than that of linkage-based localization. Genome-wide association analysis has become a popular approach to localize complex disease related genes. This approach can be performed in unrelated samples of cases and controls or in families. It requires evaluation of a large number (typically more than 500,000) of SNPs to adequately cover the genome. Unfortunately, this approach is only relevant for detecting the effects of common functional genetic variation. In cases where common genetic variants (with minor allele frequencies generally greater than 10%) underlie disease risk, genome-wide association may be more powerful than linkage for identifying genes or smaller chromosomal regions involved in a disease. Such an association-based discovery paradigm can also be performed in families as a safeguard against hidden stratification effects.

Following initial localization of potential QTLs influencing the risk of the phenotype under investigation, we can attempt to refine the chromosomal location by saturating the positional candidate region with additional genetic markers and simultaneously exploiting information on both linkage and linkage disequilibrium (whose effective signal spans a much smaller region than a linkage signal does). However, with the marker density afforded by new genome-wide genotyping technologies, this second phase of LD mapping is largely unnecessary. Instead, identification can proceed with comprehensive resequencing of those genes showing the most prior evidence for association. This resequencing step is no longer limited to the examination of common genetic variants but will also include rare functional variants that are segregating in families. Genome scanning approaches hold great promise for Chagas disease. These approaches can be used to identify the specific genes influencing risk for *T. cruzi* infection and risk for cardiac outcomes in Chagas disease.

3. EXPLORING THE GENETIC ARCHITECTURE OF CHAGAS DISEASE

3.1 Quantitative genetic methods

Quantitative genetic approaches can be used to examine multiple traits associated with *T. cruzi* infection and Chagas disease progression. For these genetic analyses, we employed SOLAR (Almasy and Blangero, 1998), a general computer package for statistical genetic analyses. These analyses permit the estimation of the heritabilities of the traits being examined. The variance component approach implemented in SOLAR can be used for both quantitative traits such as QRS intervals determined from ECGs and discrete traits such as

the presence or absence of a right bundle branch block (Williams et al., 1999). At the heart of SOLAR lies a general variance component engine that makes it possible to analyze family-based quantitative data for pedigrees of any size and complexity. Quantitative genetic analysis partitions the observed covariance among related individuals into genetic versus environmental components. Covariates such as sex, age, and their interactions were routinely included in the genetic models.

These analyses can be extended to the joint analysis of multiple traits to allow for the genetic decomposition of the observed correlations. Using the information contained in the kinship coefficients among family members and maximum likelihood variance decomposition techniques, the phenotypic correlations between any two traits can be partitioned into additive genetic and random environmental components. For example, we could examine the genetic correlation between right bundle branch block and the QT interval. The additive genetic correlation ranges between -1 and 1 and is a measure of the shared genetic basis of two traits. An absolute additive genetic correlation of 1.0 indicates complete pleiotropy (i.e., the same genes are affecting the two traits). Alternatively, a genetic correlation whose absolute value is less than 1 shows incomplete pleiotropy indicating that the two traits are influenced to some extent by the same genes but that each trait also has a genetic basis unique from the other. Similarly, the random environmental correlation serves as a measure of the strength of the correlated response of the traits to non-genetic factors. In our maximum likelihood framework, the likelihood of models that constrain the genetic correlation (or environmental correlation) between traits to zero will be compared to the likelihood of models that allow the genetic correlation (or environmental correlation) between the traits to be estimated. In this manner, we screened pairs of Chagas disease-related traits for those that are significantly genetically (and/or environmentally) correlated with one another.

We also explicitly tested for evidence of a heritable basis of the phenotypic response to infection using the quantitative genetic approach to the general examination of genotype by environment interaction developed by Blangero (1993). Seropositivity status can be considered an environment to which Chagas-related traits respond. Optimal detection of genotype by environment interaction is obtained when individuals can be experimentally manipulated to be examined in both possible environments. For the case of Chagas infection, this is clearly impossible. However, because the uninfected individuals are related to the infected individuals in our large extended pedigrees, and therefore represent the same genes exposed to two different environments, it is possible to specifically test for the genetic factors involved in differential response to seropositivity status. For this case, it is still possible to estimate the genetic variance in response to infection, although it is not possible to directly estimate the total heritability due to the absence of a statistically identifiable environmental variance due to our inability to measure any single individual both prior to infection and post-infection in a standard population-based study.

3.2 Quantitative genetics of Chagas disease-related traits: An example

As an example of genetic analyses related to Chagas disease, we examined some of our own data obtained from a pedigree-based study in Brazil. We began our work in Posse, a rural

region of the state of Goiás located approximately 350 kilometers north of Brasília in 1995. Over the last 15 years, we have collected and documented pedigree information for over 1300 individuals who have been characterized for some aspect of Chagas disease. This ongoing longitudinal study is the first large scale genetic epidemiological study of Chagas disease.

3.2.1 Heritability of Chagas disease-related traits—The detailed pedigree information available for the families participating in our studies of Chagas disease in Posse permits the estimation of heritabilities for a broad range of Chagas disease related traits. Individuals born in 1975 or earlier (i.e., at least 10 years before the vector control insecticide spraying program was initiated in the area) are all assumed to have been at risk of infection with *T. cruzi*. As noted earlier, the heritability of a trait provides an estimate of the proportion of variation in the trait attributable to genetic factors. The results of the quantitative genetic analyses of Chagas disease-related traits are provided in Table 1, along with the sample sizes for each of the traits.

Electrocardiograms were collected from participants with a portable Marquette MAC5000 System (GE Medical Systems Information Technologies, Milwaukee, WI). Seropositivity assessments were determined using three standardized tests (enzyme linked immunosorbent assay [ELISA], hemagglutination, and immunofluorescence) by the Laboratory of Cellular and Molecular Immunology at the Rene Rachou Research Center, FIOCRUZ. Individuals were considered positive if two or more of these standardized tests were positive.

Seropositivity is a discrete dichotomous trait, but it is analyzed assuming a continuous underlying distribution of liability. Seropositivity for *T. cruzi* infection is significantly heritable. The observed prevalence of seropositivity is approximately 60% among adults in the community of Posse. The heritability of seropositivity for *T. cruzi* infection in this sample of 1350 individuals born in 1975 or earlier is 0.636 indicating that approximately 64% of the observed variation in seropositivity status is attributable to genetic factors. This highly significant estimate ($p=2.2 \times 10^{-15}$) is somewhat higher than our initial estimate of 0.56 that we calculated for a smaller ($n=525$) set of individuals (Williams-Blangero et al., 1997).

We also examined several quantitative traits determined from electrocardiograms. The procedures used for the collection of the ECGs are as outlined in Williams-Blangero et al. (2007). Table 1 provides evidence that most of these phenotypes are influenced by seropositivity status as reflected in the mean difference between seropositives and seronegatives (provided by the column Infection which is given in standard deviation units). As also can be seen in Table 1, there are significant genetic components to all of the cardiovascular-related quantitative traits determined from ECG measures. The QRS interval is significantly heritable, with approximately 25% of the variation in the trait attributable to genetic factors. The QT interval shows evidence for significant genetic effects, with approximately 41% of the variation in the trait attributable to genetic factors. The PR interval is similarly heritable, with approximately 46% of the variation due to genetic effects.

We also measured blood pressure in the participants of our Chagas disease study in Posse. The blood pressure traits were also significantly heritable with about 40% of the variation in diastolic blood pressure and systolic blood pressure being attributable to genetic factors. The heritability of diastolic blood pressure was 0.414, and the heritability of systolic blood pressure was 0.365.

We also considered the potential genetic determinants of right bundle branch block, the cardiac abnormality most closely associated with Chagas disease. The presence of a right bundle branch block is significantly heritable in the Posse population, with about 54% of the variation in the trait being attributable to genetic factors.

Finally, we treated the presence of an abnormal ECG due to any reason as a phenotype in our analyses. The variation among individuals in presence of an abnormal ECG was also heritable with 38% of the variation in the trait attributable to genetic factors. The heritability of this trait was highly significant, indicating that genes do play a role in the development of cardiac outcomes in this population which is endemic for Chagas disease.

3.2.2 Shared genetic effects among Chagas disease-related traits—The

question of whether or not the genes that influence seropositivity for *T. cruzi* infection and the genes which influence the cardiac-related traits overlap can be addressed by testing for the presence of pleiotropy, i.e., genes having effects on multiple traits. There is no strong evidence for direct pleiotropy of genes jointly influencing seropositivity for *T. cruzi* infection and cardiovascular-related phenotypes, meaning that the genes which influence seropositivity status are distinct from those which determine cardiac outcomes in chronic Chagas disease. Bivariate genetic analyses were used to estimate the genetic correlation between seropositivity for *T. cruzi* infection status and each of the cardiovascular phenotypes. While all of them were in the expected direction as given by the differential mean effects shown in Table 1, none of these genetic correlations were significantly different from zero. However, both diastolic and systolic blood pressure showed negative genetic correlations (-0.156 and -0.161) which both reached suggestive ($p < 0.10$) levels of evidence indicating that there may be some overlap of genetic effects on the blood pressure related traits and the seropositivity status trait, but that a larger sample will be necessary to have sufficient power to determine this unambiguously.

These same questions of pleiotropy or genetic overlap can be addressed regarding the source of the phenotypic relationships among the cardiovascular-related traits. Table 2 shows the results of bivariate quantitative genetic analyses of the quantitative cardiovascular phenotypes. The results indicate substantial pleiotropy across some of the traits but also that there are clearly trait-specific genes acting on these phenotypes. The overall strongest evidence for the pleiotropic effects of genes is seen for the QT interval and ventricular rate which exhibit a very substantial negative genetic correlation of -0.93 ($p = 3.0 \times 10^{-17}$). Thus, about 86% ($-0.93^2 \times 100$) of the genetic variation is due to shared genetic effects between these two traits. Similarly, as expected, diastolic and systolic blood pressures exhibit a high positive genetic correlation of 0.80 ($p = 4.2 \times 10^{-10}$), suggesting that 64% of the observed genetic variation is due to the effects of pleiotropic genes.

3.2.3 Genotype by infection interaction—Table 3 presents the results of quantitative genetic tests for genotype-by-environment interaction where infection status is the environment. This statistical approach requires the availability of relatives in each of the two environments. In the present case, the environments are: 1) the state of being seropositive for *T. cruzi* infection and 2) the state of not being seropositive for infection. Our large pedigrees allow such contrasts to be made. As described by Blangero (1993), a significant G×E interaction is evidence for a heritable response to the environment. There are two possible tests for such interaction. The first is a test for differences in the additive genetic variance for the trait as expressed in each environment. When there is a difference in genetic variance, there is obligate evidence for the heritability of the response to the environment.

Table 3 shows that three of the cardiovascular traits (the QRS interval, systolic blood pressure and ventricular rate) show significant evidence for different additive genetic variances in individuals seropositive for *T. cruzi* infection as compared to seronegative individuals. For both the QRS interval and ventricular rate, seropositive individuals express larger amounts of genetic variance than do seronegatives. However, for systolic blood pressure, this is reversed. The second test for genotype-by-environment interaction examines whether the additive genetic correlation (ρ_G) between the trait's expression in the two environments is different from 1. When $\rho_G \neq 1$, there is evidence for different genes influencing the expression of the trait in the two environments which again induces obligate heritability for the response to the environment. In Table 3, one important trait, the presence or absence of an abnormal ECG, provides significant evidence for such G×Infection interaction with an estimated genetic correlation of 0.36 between the environments. This suggests that there are different genes acting on the risk of abnormal ECG in infected individuals versus uninfected individuals. The QRS interval provides suggestive evidence for such interaction ($\rho_G = 0.50$, $p=0.081$).

Overall, our quantitative genetic analyses provide evidence that the cardiovascular response to seropositivity for *T. cruzi* infection is heritable for at least four traits (QRS interval, systolic blood pressure, ventricular rate, and abnormal ECG). Because such tests provide lower power than a direct longitudinal assessment of change in these characters, the absence of such an interaction should not be counted as evidence against a genetic basis of the response to infection. Our ongoing mixed longitudinal collection of cardiovascular phenotypes in infected individuals will ultimately provide a more powerful basis for the formal test of the role of genetic factors in the cardiovascular response to *T. cruzi* infection.

4. MAPPING THE FUTURE OF GENETIC EPIDEMIOLOGICAL STUDIES OF CHAGAS DISEASE

We have presented an overview of past and ongoing assessments of host genetic factors in Chagas disease. To date, the focus has been on the demonstration that host factors in both susceptibility to *T. cruzi* infection and cardiovascular response to infection have heritable components. The task of localization of the underlying QTLs has just begun. In our study in Brazil, we are currently completing a genome-wide high density SNP screen with over 1M SNPs. Because we are studying very large pedigrees, we will be able to jointly utilize both

linkage and association information in order to cover the complete functional allelic spectrum from rare to common. However, this joint linkage/association approach will also be limited to the genomic localization of the underlying QTLs. Actual gene identification will require a deep-sequencing approach in which all of the sequence variation is evaluated in the regions of localization and intensive genetic work performed to identify both causal variants and causal genes. The advent of high throughput Next Generation Sequencing (NGS) should make gene identification feasible. Indeed, we are soon approaching the day when we will be able to economically obtain whole genome sequence data on our subjects. This should allow a direct search for the functional variants involved in both susceptibility to Chagas disease and the cardiovascular response to infection. Large pedigrees will be particularly useful if the underlying variation is rare. Such pedigrees allow for the examination of multiple copies of even private founder variants. Therefore, founder lineage size should be maximized in order to be able to examine both the effects of common and rare variants. Additionally, as seen in our analyses above, tests for genotype $\times$ environment interaction are also optimal in large families since relatives can often be observed in both environments.

ACKNOWLEDGEMENTS

This work was supported by a grant (R01 HL089849) from the National Institutes of Health. Statistical genetics support was provided by R01 MH059490. We thank Antonio R.L. Teixeira for his contributions to initiating the research effort in Posse. We are also grateful to the people of Posse for their generous cooperation with this study.

REFERENCES

- Aguilar HM, Abad-Franch F, Pinto Dias JC, Junqueira ACV, and Coura JR (2007). Chagas disease in the Amazon region. *Memórias do Instituto de Oswaldo Cruz* 102:47–55.
- Almasy LA and Blangero J (1998). Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics* 62:1198–1211. [PubMed: 9545414]
- Añez N, Crisante G, Maia da Silva F, Rojas A, Carrasco H, Umezawa WS, Stolf AMS, Ramirez JL, and Teixeira MMG (2004) Predominance of lineage I among *Trypanosoma cruzi* isolates from Venezuelan patients with different clinical profiles of acute Chagas disease. *Tropical Medicine and International Health* 9:1319–1326. [PubMed: 15598264]
- Barbosa CAA, Morton NE, Rao DC, and Krieger H (1981). Biological and cultural determinants of immunoglobulin levels in a Brazilian population with Chagas' disease. *Human Genetics* 59:161–163. [PubMed: 6799387]
- Beard CB, Pye G, Steurer FJ, Rodrigues R, Campman R, Peterson AT, Ramsey J, Wirtz RA, and Robinson LE (2003). Chagas disease in a domestic transmission cycle, southern Texas, USA. *Emerging Infectious Diseases* 9:103–105. [PubMed: 12533289]
- Bern C, and Montgomery SP (2009). An estimate of the burden of Chagas disease in the United States. *Clinical Infectious Diseases* 49:e52–254. [PubMed: 19640226]
- Biolo A, Ribeiro AL and Clausell N (2010). Chagas cardiomyopathy – where do we stand after a hundred years? *Progress in Cardiovascular Diseases* 52:300–316. [PubMed: 20109600]
- Blackwell JM, Jamieson SE, and Burgner D (2009). HLA and infectious diseases. *Clinical Microbiology Reviews* 22:370–385. [PubMed: 19366919]
- Blangero J (1993). Statistical genetic approaches to human adaptability. *Human Biology* 65:941–946. [PubMed: 8300087]
- Borges JS, Machada de Assis GF, Gomes LV, Dias JCP, Pinto IDM, Martins-Filho OA, Torres RM, Vinas PA, Bahia MT, Machado-Coelho GLL, and Lana M (2006). Seroprevalence of Chagas disease in school children from two municipalities of Jequitinhonha Valley, Minas Gerais, Brazil:

Six years following the onset of epidemiological surveillance. *Revista do Instituto de Medicina Tropical da São Paulo* 48:81–86.

- Briceño-Leon R (2007). Chagas disease and globalization of the Amazon. *Cadernos de Saúde Pública* 23(Supplement 1):S33–S40. [PubMed: 17308715]
- Buscaglia CA, and Di Noia JM (2003). *Trypanosoma cruzi* clonal diversity and the epidemiology of Chagas disease. *Microbes and Infection* 5:419–427. [PubMed: 12737998]
- Busch MP, Kleinman SH, and Nemo GJ (2003). Current and emerging infectious risks of blood transfusions. *Journal of the American Medical Association* 289:959–961. [PubMed: 12597733]
- Calzada JE, Pineda V, Garisto JD, Samudio F, Santamaria AM, and Saldaña A (2010). Human trypanosomiasis in the eastern region of the Panama province: New endemic areas for Chagas disease. *American Journal of Tropical Medicine and Hygiene* 82:580–582. [PubMed: 20348502]
- Campbell DA, Westenberger SJ, and Strum NR (2004). The determinants of Chagas disease: Connecting parasite and host genetics. *Current Molecular Medicine* 4:549–562. [PubMed: 15357207]
- Castro JA, de Mecca MM, and Bartel LC (2006). Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). *Human Experimental Toxicology* 25:471–479. [PubMed: 16937919]
- Coll-Cardenas R, Espinoza-Gomez F, Maldonado-Rodriguez A, Reyes-Lopez PA, Huerta-Viera M, Rojas-Larios F (2004). Active transmission of human Chagas disease in Colima Mexico. *Memórias do Instituto Oswaldo Cruz* 99:363–368. [PubMed: 15322624]
- Colorado IA, Acquatella H, Cataliotti F, Fernandez MT, and Layrisse Z (2000). HLA class II DRB1, DPB1 polymorphisms and cardiomyopathy due to *Trypanosoma cruzi* chronic infection. *Human Immunology* 61:320–325. [PubMed: 10689123]
- Cruz-Robles D, Reyes PA, Monteon-Padilla VM, Ortiz-Muniz AR, and Varga-Alarcon G (2004). MHC class I and class II genes in Mexican patients with Chagas disease. *Human Immunology* 65:60–65. [PubMed: 14700597]
- Cruz-Robles D, Chávez-González JP, Cavazos-Quero MM, Pérez-Méndez O, Reyes PA, and Vargas-Alarcón G (2009). Association between IL-1B and IL-1RN gene polymorphisms and Chagas' disease development susceptibility. *Immunological Investigations* 38:231–239. [PubMed: 19811434]
- Drigo SA, Cunha-Neto E, Ianni B, Cardoso MR, Braga PE, Fae FC, Nunes VL, Buck P, Mady C, Kalil J, and Goldberg AC (2006). TNF gene polymorphisms are associated with reduced survival in severe Chagas disease cardiomyopathy patients. *Microbes and Infection* 8:598–603. [PubMed: 16427798]
- Drigo SA, Cunha-Neto E, Ianni B, Mady C, Faé KC, Buck P, Kalil J, and Goldberg AC (2007). Lack of association of tumor necrosis factor- α polymorphisms with Chagas disease in Brazilian patients. *Immunology Letters* 108:109–111. [PubMed: 17141882]
- Dutra WO, Rocha MOC, and Teixeira MM (2005). The clinical immunology of Chagas disease. *Trends in Parasitology* 21:581–587. [PubMed: 16236550]
- Fernandez-Mestre MT, Layrisse Z, Mantagnani S, Acquatella H, Cataliotti F, Matos M, Blabas O, Makhatadze N, Dominguez E, Herrera F, and Madrigal A (1998). Influence of HLA class II polymorphism in chronic Chagas disease. *Parasite Immunology* 20:197–203. [PubMed: 9618730]
- Flórez O, Zafra G, Morillo C, Martín J, and González CI (2006). Interleukin-1 gene cluster polymorphism in Chagas disease in a Colombian case-control study. *Human Immunology* 67:741–748. [PubMed: 17002905]
- García Borrás S, Racca L, Cotorruelo C, Biondi C, Beloscar J, and Racca A (2009). Distribution of HLA-DRB1 alleles in Argentinean patients with Chagas disease cardiomyopathy. *Immunological Investigations* 38:268–275. [PubMed: 19811437]
- Garcia-Zapata MTA, and Marsden PD (1986). Chagas disease. *Clinical Tropical Medicine and Communicable Disease* 1:557–585.
- Garg N and Bhatia V (2005). Current status and future prospects for a vaccine against American trypanosomiasis. *Expert Reviews in Vaccines* 4:867–880.

- Goldbaum M, Ajimura FY, Litvoc J, Alves de Carvalho S, and Eluf-Neto J (2004). American trypanosomiasis and electrocardiographic alterations among industrial works in São Paulo Brazil. *Revista do Instituto de Medicina Tropical da São Paulo* 46:299–302.
- Grijalva MJ, Escalante L, Paredes RA, Costales JA, Padilla A, Rowland EC, Aguilar JM, and Racines J (2003). Seroprevalence and risk factors for *Trypanosoma cruzi* infection in the Amazon region of Ecuador. *American Journal of Tropical Medicine and Hygiene* 69:380–385. [PubMed: 14640497]
- Gurtler RE, Cecere MC, Rubel DN, and Schiewegmann NJ (1992). Determinants of domiciliary density of *Triatoma infestans*, vector of Chagas disease. *Medical and Veterinary Entomology* 6:75–83. [PubMed: 1600232]
- Jorge MT, Macedo TA, Janones RS, Carizzi DP, Heredia RA, and Acha RE (2003). Types of arrhythmia among cases of American trypanosomiasis compared with those in other cardiology patients. *Annals of Tropical Medicine and Parasitology* 97:139–148. [PubMed: 12803869]
- Kirchhoff LV (1999) American trypanosomiasis (Chagas' disease). In: *Tropical Infectious Diseases: Principles, Pathogens, and Practice*, Guerrant RL, Walker DH, and Weller PF (eds). New York: Churchill Livingstone Pp. 785–796.
- Layrisse Z, Fernandez MT, Montagnani D, Matos M, Balbas O, Herrera F, Colorado IA, Cataliotti F, and Acquatella H (2000). HLA-C(*)03 is a risk factor for cardiomyopathy in Chagas disease. *Human Immunology* 61:925–929. [PubMed: 11053636]
- Leiby DA, Herron RM, Read EJ, Lenes BA, and Stumpf RJ (2002). *Trypanosoma cruzi* in Los Angeles and Miami blood donors: Impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. *Transfusion* 42:549–555. [PubMed: 12084162]
- Lescure FX, LeCoup G, Freilij H, Develoux M, Paris L, Brutus L, Pialoux G (2010). Chagas disease: Changes in knowledge and management. *Lancet Infectious Diseases* 10:556–570. [PubMed: 20670903]
- Llop ER, Rothhammer F, Acuña M, Apt W, Arribada A (1991). Antígenos HLA en cariopatas chagasicos: Nueva evidencia basada en un analisis de casos y controles. *Revista Medica de Chile* 119:633–636. [PubMed: 1844366]
- Macedo AM, Machado CR, Oliveira RP, and Pena SDJ (2004). *Trypanosoma cruzi*: Genetic structure of populations and relevance of genetic variability to the pathogenesis of Chagas disease. *Memórias do Instituto Oswaldo Cruz* 99:1–12.
- Maguire JH, Hoff R, Sherlock I, Guimaraes AC, Sleigh AC, Ramos NB, Mott KE, and Weller TH (1987). Cardiac morbidity and mortality due to Chagas disease: Prospective electrocardiographic study of a Brazilian community. *Circulation* 75:1140–1145. [PubMed: 3552307]
- Manoel-Caetano FS, and Silva AE (2007). Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease. *Cadernos Saúde Pública*, Rio de Janeiro 23:2263–2274.
- McCarthy M (2003). American Red Cross to screen blood for Chagas disease. *Lancet* 362:1988.
- Miles MA, Llewellyn MS, Lewis MD, Yeo M, Baleela R, Fitzpatrick S, Gaunt MW, and Mauricio IL (2009). The molecular epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*: Looking back and to the future. *Parasitology* 136:1509–1528. [PubMed: 19691868]
- Moreno M, Silva EL, Ramirez LE, Palacio LG, Rivera D, and Arcos-Burgos M (2004). Chagas disease susceptibility/resistance: Linkage disequilibrium suggests epistasis between major histocompatibility complex and interleukin 10. *Tissue Antigens* 64:18–24. [PubMed: 15191519]
- Mott KE, Lehman JS Jr., Hoff R, Morrow RH, Muniz TM, Sherlock I, Draper CC, Pugliese C, and Guimaraes AC (1976). The epidemiology and household distribution of seroreactivity to *Trypanosoma cruzi* in a rural community in northeast Brazil. *American Journal of Tropical Medicine and Hygiene* 25:552–562. [PubMed: 822736]
- Nieto A, Beraun Y, Collado MD, Caballero A, Alonso A, Gonzalez A, and Martin J (2000). HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection. *Tissue Antigens* 55:195–198. [PubMed: 10777093]
- Norman FF, Pérez de Ayala A, Pérez-Molina JA, Monge-Maillo B, Zamarrón P, and López-Vélez R (2010). Neglected tropical diseases outside the tropics. *PLoS Neglected Tropical Diseases* 4:e762. [PubMed: 20668546]

- Ohlstein WH, Ruffolo RR Jr., and Elliott JD (2000). Drug discovery in the next millennium. *Annual Review of Pharmacology and Toxicology* 40:177–191.
- Pinto AYN, Valente SAS, and Valente VC (2004). Emerging acute Chagas disease in Amazonian Brazil: Case reports with serious cardiac involvement. *Brazilian Journal of Infectious Disease* 8:454–460.
- Prata A (2001). Clinical and epidemiological aspects of Chagas' disease. *Lancet Infectious Diseases* 1:92–100. [PubMed: 11871482]
- Ramasawmy R, Cunha-Neto E, Fae KC, Martello FG, Müller NG, Cavalcanti VL, Ianni B, Mady C, Kalil J, and Goldberg AC (2006). The monocyte chemoattractant protein-1 gene polymorphism is associated with cardiomyopathy in human chagas disease. *Clinical Infectious Diseases* 43:305–311. [PubMed: 16804844]
- Ramasawmy R, Fae KC, Cunha-Neto E, Müller NG, Cavalcanti VL, Ferreira RC, Drigo SA, Ianni B, Mady C, Goldberg AC, and Kalil J (2007). Polymorphisms in the gene for lymphotoxin-alpha predispose to chronic Chagas cardiomyopathy. *Journal of Infectious Diseases* 196:1836–1843. [PubMed: 18190265]
- Ramasawmy R, Fae KC, Cunha-Neto E, Borba SC, Ianni B, Mady C, Goldberg AC, and Kalil J (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. *Molecular Immunology* 45:283–288. [PubMed: 17544510]
- Ramasawmy R, Cunha-Neto E, Fae KC, Borba SCP, Teixeira PC, Ferreira SCP, Goldberg AC, Ianni B, Mady C, and Kalil J (2009). Heterozygosity for the A180L 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. *Journal of Infectious Diseases* 199:1838–1845. [PubMed: 19456234]
- Rangel-Flores H, Sánchez B, Mendoza-Duarte J, Barnabé C, Brenière FS, Ramos C, and Espinoza B (2001). Serologic and parasitologic demonstration of *Trypanosoma cruzi* infections in an urban area of central Mexico: Correlation with electrocardiographic alterations. *American Journal of Tropical Medicine and Hygiene* 65:887–895. [PubMed: 11791993]
- Rassi A Jr., Rassi A, and Marin-Neto JA (2010) Chagas disease. *Lancet* 375:1388–1402. [PubMed: 20399979]
- Rizzo NR, Arana BA, Diaz A, Cordon-Rosales C, Klein RW, and Powell MR (2003). Seroprevalence of *Trypanosoma cruzi* infection among school-age children in the endemic area of Guatemala. *American Journal of Tropical Medicine and Hygiene* 68:678–682. [PubMed: 12887026]
- Rodriguez Coura J, and Castro S.L. de (2002). A critical review on Chagas disease chemotherapy. *Memórias do Instituto Oswaldo Cruz* 97:3–24. [PubMed: 11992141]
- Salles G, Xavier S, Sousa A, Hasslocher-Moreno A, and Cardosa C (2003). Prognostic value of QT interval parameters for mortality risk stratification in Chagas disease: Results of a long-term follow-up study. *Circulation* 108:305–312. [PubMed: 12835217]
- Salles G, Xavier S, Sousa A, Hasslocher-Moreno A, and Cardosa C (2004). T-wave axis deviation as an independent predictor of mortality in chronic Chagas disease. *American Journal of Cardiology* 93:1136–1140. [PubMed: 15110206]
- Sanchez-Sancho F, Campillo NE, and Páez JA (2010). Chagas disease: Progress and new perspectives. *Current Medicinal Chemistry* 17:423–452. [PubMed: 20015038]
- Schadt EE, Monks SA, and Friend SH (2003). A new paradigm for drug discovery: Integrating clinical, genetic, genomic, and molecular phenotype data to identify drug targets. *Drug Discovery and Design* 31:437–443.
- Sarkar S, Strutz SE, Frank DM, Rivaldi CL, Sissel N, and Sanchez-Cordero V (2010). Chagas disease risk in Texas. *PLoS Neglected Tropical Diseases* 4:e836. [PubMed: 20957148]
- Silva-Grecco RL, Balarin MA, Correia D, Prata A, and Rodrigues V Jr. (2010). Familial analysis of seropositivity to *Trypanosoma cruzi* and of clinical forms of Chagas disease. *American Journal of Tropical Medicine and Hygiene* 82:45–48. [PubMed: 20064994]
- Sosa-Jurado F, Mazariego-Aranda M, Hernandez-Becerril N, Garza-Murillo V, Cardenas M, Reyes PA, Hirayama K, Monteón VM (2003). Electrocardiographic findings in Mexican Chagasic subjects

- living in high and low endemic regions of *Trypanosoma cruzi* infection. *Memórias do Instituto Oswaldo Cruz* 98:605–610. [PubMed: 12973525]
- Sturm NR, Vargas NS, Westenberger SJ, Zingales B, and Campbell DA (2003). Evidence for multiple hybrid groups in *Trypanosoma cruzi*. *International Journal of Parasitology* 33:269–279. [PubMed: 12670512]
- Tanowitz HB, Machado FS, Jelicks LA, Shirani J, Campos de Carvalho AC, Spray DC, Factor SM, Kirchhoff LV, and Weiss LM (2009). Perspectives on *Trypanosoma cruzi*-induced heart disease (Chagas disease). *Progress in Cardiovascular Diseases* 51:524–539. [PubMed: 19410685]
- Teixeira ARL, Cordoba JC, Maior IS, and Solorzano E (1990a). Chagas disease: Lymphoma growth in rabbits treated with benznidazole. *American Journal of Tropical Medicine and Hygiene* 43:146–158. [PubMed: 2117856]
- Teixeira ARL, Silva R, Neto EC, Santano JM, and Rizzo LV (1990b). Malignant, non-Hodgkin's lymphoma in *Trypanosoma cruzi* infected rabbits treated with nitroarenes. *Journal of Comparative Pathology* 103:37–48. [PubMed: 2203830]
- Tibayrenc M (1998). Integrated genetic epidemiology of infectious diseases: The Chagas model. *Memórias do Instituto Oswaldo Cruz* 93:577–580. [PubMed: 9830520]
- Tibayrenc M (1999). Toward an integrated genetic epidemiology of parasitic protozoa and other pathogens. *Annual Review of Genetics* 33:449–477.
- Tibayrenc M (2007). Human genetic diversity and the epidemiology of parasitic and other transmissible diseases. *Advances in Parasitology* 64:377–422. [PubMed: 17499103]
- Tibayrenc M (2010). Modelling the transmission of *Trypanosoma cruzi*: The need for an integrated genetic epidemiological and population genomics approach. *Advances in Experimental Medicine and Biology* 673:200–211. [PubMed: 20632539]
- Torres OA, Calzada JE, Beraún Y, Morillo CA, González A, González CI, and Martin J (2010). Role of the IFNG +874T/A polymorphism in Chagas disease in a Colombian population. *Infection, Genetics, and Evolution* 10:682–685.
- Urbina JA, and Docampo R (2003). Specific chemotherapy of Chagas disease: Controversies and advances. *Trends in Parasitology* 19:495–501. [PubMed: 14580960]
- WHO (2010) Chagas (American trypanosomiasis) fact sheet (revise June 2010). *Weekly Epidemiological Record* 85(34):334–336. [PubMed: 20726172]
- Williams JT, Van Eerdewegh P, Almasy L, and Blangero J (1999). Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. *American Journal of Human Genetics* 65:1134–1147. [PubMed: 10486333]
- Williams-Blangero S, and Blangero J (2006). Collection of pedigree data for genetic analysis in isolate populations. *Human Biology* 78:89–101. [PubMed: 16900884]
- Williams-Blangero S, VandeBerg JL, Blangero J, and Teixeira AR (1997). Genetic epidemiology of seropositivity for *Trypanosoma cruzi* infection in rural Goiás, Brazil. *American Journal of Tropical Medicine and Hygiene* 57:538–543. [PubMed: 9392592]
- Williams-Blangero S, Magalhaes T, Rainwater E, Blangero J, Corrêa-Oliveira R, and VandeBerg JL (2007). Electrocardiographic characteristics in a population with high rates of seropositivity for *Trypanosoma cruzi* infection. *American Journal of Tropical Medicine and Hygiene* 77:495–499. [PubMed: 17827366]
- Yacoub S, Birks EJ, Slavik Z, and Henein M (2003). Early detection of myocardial dysfunction in Chagas disease using novel echocardiographic indices. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 97:528–534. [PubMed: 15307418]
- Zicker F, Smith PG, Netto JC, Oliveira RM, Zicker EM (1990). Physical activity, opportunity for reinfection, and sibling history of heart disease as risk factors for Chagas' cardiomyopathy. *American Journal of Tropical Medicine and Hygiene* 43:498–505. [PubMed: 2240374]

Table 1.

Heritabilities for *T. cruzi* seropositivity, cardiovascular phenotypes, and the effects on infection.

Trait	N	h^2	$p(h^2)$	Infection (SDU)	$p(\text{infect})$
<i>T. cruzi</i> Seropositivity	1350	0.636	2.2×10^{-15}	----	----
QRS (ms)	1190	0.250	9.0×10^{-7}	0.344	3.5×10^{-8}
QT (ms)	1199	0.411	3.5×10^{-20}	0.417	5.2×10^{-12}
PR (ms)	1168	0.458	5.7×10^{-17}	0.095	0.136
Ventricular Rate (bpm)	1190	0.386	3.9×10^{-17}	-0.244	1.2×10^{-4}
Diastolic BP (mmHg)	1104	0.414	2.1×10^{-15}	-0.250	1.1×10^{-4}
Systolic BP (mmHg)	1104	0.365	5.2×10^{-13}	-0.272	1.6×10^{-5}
Right Bundle Branch Block	1190	0.536	4.1×10^{-4}	1.301	7.5×10^{-12}
Abnormal ECG	1190	0.377	5.6×10^{-5}	0.577	5.1×10^{-10}

Table 2.

Genetic correlations between quantitative cardiovascular phenotypes.

Trait	Trait	ρ_G	$p(\rho_G = 0)$
QT	QRS	0.59	3.1×10^{-6}
QT	PR	0.21	0.0757
QT	Ventricular Rate	-0.93	3.0×10^{-17}
QT	Diastolic BP	-0.28	0.0169
QT	Systolic BP	-0.14	0.2623
QRS	PR	0.28	0.0534
QRS	Ventricular Rate	-0.48	0.0013
QRS	Diastolic BP	0.21	0.1609
QRS	Systolic BP	0.24	0.1294
PR	Ventricular Rate	-0.22	0.0808
PR	Diastolic BP	0.17	0.1687
PR	Systolic BP	0.22	0.0804
Ventricular Rate	Diastolic BP	0.44	0.0004
Ventricular Rate	Systolic BP	0.25	0.0521
Diastolic BP	Systolic BP	0.80	4.2×10^{-10}

Table 3.

Tests of genotype-by-infection interaction suggesting a genetic basis of response to infection.

Trait	Observed Genetic Variance	p(equal σ^2_G)	ρ_G	p($\rho_G = 1$)
QRS	Seropos > Seroneg	0.050	0.50	0.081
QT	Seropos = Seroneg	0.369	0.99	0.493
PR	Seropos = Seroneg	0.391	1	0.500
Ventricular Rate	Seropos > Seroneg	0.035	0.93	0.338
Diastolic BP	Seropos = Seroneg	0.748	1	0.500
Systolic BP	Seropos > Seroneg	0.042	1	0.500
Right Bundle Branch Block	Seropos = Seroneg	0.799	0.42	0.338
Abnormal ECG	Seropos = Seroneg	0.856	0.36	0.044