[Frontiers in Bioscience 8, e337-345, May 1, 2003]

GENETIC EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* INFECTION AND CHAGAS' DISEASE

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1. ABSTRACT

Chagas' disease is a leading cause of heart disease throughout Latin America, affecting an estimated 16 to 18 million individuals. Given the large pool of primary hosts for this zoonotic disease, complete eradication of Chagas' disease through control of the arthropod vector is unlikely. Research with both humans and animal models indicates that there is considerable variation in susceptibility to infection and disease outcome, and that this variation may be due in part to genetic factors. This paper summarizes the evidence for genetic control of susceptibility to *Trypanosoma cruzi* infection and severity of disease outcome in Chagas' disease. The lack of an effective treatment or prevention for Chagas' disease indicates the great potential for genetic studies, and particularly for genome scans of extended human pedigrees, to improve our understanding of the determinants of this complex disease, and ultimately to suggest new pathways to be targeted in drug development efforts.

2. INTRODUCTION

Chagas' disease, like many parasitic diseases, remains a significant global health burden and still lacks effective pharmacological preventions or interventions. Genetic epidemiological studies can suggest new biological pathways to target in drug development efforts aimed at preventing and treating parasitic diseases (1,2). There is substantial evidence for the role of host genetic factors in a broad range of these important infections (3-9), and individual genes influencing susceptibility already have been localized for schistosomiasis and ascariasis (9, 10). The genetic approaches that have successfully characterized the genetic determinants of other common parasitic diseases are also appropriate for the study of *Trypanosoma cruzi* infection and Chagas' disease.

Chagas' disease is found in almost every country in South and Central America (11). The parasite is transmitted to humans by reduviid bugs (genera *Triatoma, Rhodnius,* and *Panstrongylus*) when feces deposited after a blood meal come in direct contact with mucosa, conjunctiva, or open wounds in the host (12-13). Active vector control programs have been implemented throughout Latin America, but despite these efforts the disease remains the leading cause of heart disease in that area of the world (11, 14). While transmission of new infections has been reduced, an estimated 16-18 million individuals are currently infected and over a hundred million are considered at risk for infection (15-17).

There is a tremendous health and economic cost associated with Chagas' disease, and the current number of *T. cruzi* infected individuals indicates that the disease will continue to be a significant health burden for at least the next fifty years even if vector control efforts continue (16, 18). This is because a substantial proportion of the people already infected with *T. cruzi* who do not have clinical disease, are likely to develop serious health consequences in the future. For example, it is estimated that in Brazil 75,000 people per year develop cardiac arrhythmias associated with Chagas' disease and that the costs of pacemaker implantation and surgery associated with Chagasic cardiomyopathy are in excess of $375 million per year (16). The World Bank's estimate of the tremendous disability and associated lost adjusted years of life, makes the impact of Chagas' disease third among all tropical diseases, with only malaria and schistosomiasis exerting greater tolls (16, 19).

T. cruzi infection has been demonstrated in Chilean mummies dating from 2000 BC indicating that Chagas' disease has been a persistent health threat in South

America since prehistoric times (20-23). While vector control programs have achieved local success, the zoonotic disease is unlikely to be permanently eradicated by this means because the pool of primary hosts is large and several species of reduviid bugs can transmit the parasite (24). If vector control programs are stopped after transmission rates decline to negligible levels, a gradual reinfestation of homes is possible and transmission to humans could begin again. New infections occur even in countries with active control programs, especially when people migrate and settle in new geographic areas that have populations of infected triatomines (e.g., 25-28). In addition to the questionable feasibility of completely eliminating the infected vectors, there is now evidence of incipient insecticide resistance developing in the bugs (29-30). Thus, while infection rates have greatly diminished the potential for new infections with *T. cruzi* should not be underestimated.

The lack of a vaccine or effective drugs to prevent or treat chronic Chagas' disease demands that there be more research to facilitate development of new effective pharmacologic agents for prevention of infection and treatment of this disease (31-33). The identification of the specific genetic factors influencing this complex disease entity could yield important new information about biological mechanisms to be targeted in drug development efforts to improve prevention and intervention in this disease.

3. PHENOTYPES ASSOCIATED WITH CHAGAS' DISEASE

Genetic epidemiological studies of Chagas' disease require clear definition of the phenotype to be analyzed. Chagas' disease is a complex entity, involving several stages, different clinical types, and many correlates of disease that may be subjected to genetic analysis. The initial step in the development of Chagas' disease is infection with the parasitic organism, *Trypanosoma cruzi*. Subsequent progression to Chagas' disease generally occurs in two phases. First, there is an acute phase, lasting 2-3 months following parasitic infection, that is sometimes associated with the formation of a characteristic swelling or a chagoma at the site of infection (34-35). In stable populations living in endemic areas, acute Chagas' disease is generally a pediatric disease. Many individuals are asymptomatic throughout the acute phase of the illness, although between 5% and 10% of individuals experience severe or even fatal consequences during the acute phase (34). About 40% of those infected recover spontaneously from the acute phase, and remain seropositive but disease-free for the remainder of their lives, even though they may have electrocardiographic abnormalities.

In the remaining 60% of cases the disease progresses to a chronic phase characterized by progressive cardiomyopathy in the majority of cases, or by megacolon and/or megaesophagus in about 3% of cases (34, 36). Individuals with the gastrointestinal form of the disease frequently also present cardiac symptoms. Longitudinal human population studies that include assessments of children indicate that the cardiac abnormalities associated with chronic infection develop quite early in life even though clinical signs generally do not appear until adulthood (37-38).

The most commonly used method to assess the cardiac lesions associated with Chagas' disease is electrocardiography (39). The cardiac form of Chagas' disease is evidenced in electrocardiograms by conduction abnormalities including bradyarrhythmias, premature ventricular contractions, and bundle-branch block, particularly right bundle-branch block (13, 14, 40). The rarer digestive form is characterized by development of megacolon and/or megaesophagus with symptoms that may include abdominal pain, chronic constipation with the maximum number of days between bowel movements being 10 (71% of cases of megacolon) to 30 or more (14% of cases of megacolon), difficulty swallowing, gastroesophageal reflux, and chest pain not related to cardiac disease (13, 41-42). Just as asymptomatic individuals can exhibit cardiac abnormalities, seropositive individuals may have subclinical abnormalities related to the digestive form of the disease (43).

One of the major questions that has challenged investigators in the field of Chagas' disease is what determines the outcome of the severe clinical form of the disease. The fact that the development of morbidity in Chagas' disease occurs long after the initial infection has led to the search for markers that can predict development of the severe forms of the disease (44). These correlates of disease may themselves have important genetic determinants. Characterizing the genetic factors that determine predictors of disease progression can allow identification of the individuals most likely to progress to severe disease, and thereby provide a means of targeting limited medical resources to those in greatest need.

Cell-mediated immunity is undoubtedly of major importance in the development of all severe clinical forms of Chagas' disease. In this context, recent reports strongly support a role for cytotoxic immune mechanisms in the development of severe Chagas' disease (45-46). Response to *T. cruzi* infection has most commonly been found to be a Th 1 biased response (but see (40)) associated with high interferon gamma levels (48-51). This finding has led researchers to speculate that the cardiomyopathy associated with chronic *T. cruzi* infection may be due to a long-term inflammatory response to persistent parasitism rather than an autoimmune process (52). The presence of parasite derived DNA in relevant tissue has been demonstrated for both the cardiac and gastrointestinal forms of the disease (45, 53-54). However, evidence documenting cellular and humoral reactivity to heart tissue components has also been published (47, 55).

Studies using flow cytometry have demonstrated that antibody responses can also be correlated with the development of severe forms of the disease or cure after specific treatment (56-58). Although several studies have

demonstrated the importance of the cellular immune response on the development of the severe cardiac lesion, little is known about the role of soluble factors on the development of this pathology (45, 55, 59).

4. GENETIC FACTORS ASSOCIATED WITH RISK OF CHAGAS' DISEASE IN HUMAN POPULATIONS

Epidemiological studies of *T. cruzi* infection and Chagas' disease in many human populations have suggested a probable role for genetic factors in determining differential susceptibility to infection and to differential disease progression subsequent to infection due to the frequently observed familial clustering of disease (60-61). Feitosa and Krieger developed a mathematical model to describe the distribution of Chagas' disease observed in northwestern Brazil (62). This model was based on a double binomial with one tail excess and fit the epidemiological data well, suggesting that a small percentage of families were at high risk for infection with *T. cruzi*. The authors attributed this clustering of risk to environmental factors associated with risk of exposure to the triatomine bug vector, but the result is also consistent with certain families having an increased genetic susceptibility to infection. A case-control study of Chagas' disease patients and normal individuals found a positive effect of sibling history of heart disease on risk of Chagas' disease, leading the authors to conclude that there is evidence for familial aggregation of Chagas' disease (63). However, a study of seropositive sibships conducted in Argentina found no evidence for a familial patterning to electrocardiographic abnormalities in individuals infected with *T. cruzi* (64).

It has been speculated that familial clustering of Chagas' disease may be due to genetic factors, including the HLA system, which influence susceptibility to infection (34, 65-66). Several studies have documented an association between HLA alleles and seronegativity for *Trypanosoma cruzi* in populations assumed to have high rates of exposure to the vector and the parasitic organism. Fernandez-Mestre and colleagues found an association between HLA class II alleles and seronegativity for *T. cruzi* in a population with endemic disease from Venezuela (67). Deghaide and colleagues found an association between HLA class II alleles and lack of seropositivity to *T. cruzi* in a Brazilian population and suggested that an HLA class I allele associated with seropositivity was associated with susceptibility to infection (68). Investigators working in Peru, demonstrated an association between HLA class II alleles and seronegativity status in a population from an endemic area (66).

There have been a number of studies evaluating the role of HLA in determining differential disease outcomes in chronic *T. cruzi* infection among seropositive individuals. Many of these reported an association between HLA haplotypes and disease progression (67, 69-71). For example, Colorado and colleagues found an association between HLA class II haplotypes and the development of cardiomyopathy in *T. cruzi* infected individuals in their assessment of 111 seropositive patients from Venezuela who ranged from having no symptoms to having severe congestive heart failure (70). An evaluation of 113 seropositive individuals by this same research group also documented an association between HLA class I alleles and cardiomyopathy (71). Similarly, Llop and colleagues found that HLA class I haplotypes were associated with severity of cardiomyopathy in a sample of seropositive Chilean individuals (69). Comparing seropositive individuals to a sample of uninfected people, Fernandez-Mestre and colleagues found that certain HLA class II genes were associated with the arrythmias and congestive heart failure in 67 affected individuals as compared to 156 controls (67).

Despite the numerous findings of associations between various HLA alleles and haplotypes and clinical progression of Chagas' disease, there is little consistency among studies in the specific alleles and haplotypes identified as associated with differential outcome of long-term infection with *T. cruzi*. In addition to this lack of consistency among positive results, there are a number of reports that show no association between HLA genes and severity of cardiac disease in *T. cruzi* infected patients. For example, an assessment of asymptomatic seropositive patients and *T. cruzi* infected individuals experiencing cardiac sequelae of Chagas' disease from a clinic population in Brazil found no association between HLA class II alleles and cardiomyopathy (72). A study of asymptomatic and cardiac-impaired chronically infected individuals in Venezuela documented no association between HLA class I or HLA class II alleles and disease outcome in individuals with *T. cruzi* infection (73). A study of 85 seropositive individuals from a rural Peruvian population revealed no association between HLA class II genes and cardiac symptoms of Chagas' disease (66).

The examinations of HLA effects have been based on relatively small sample sizes and have the many problems inherent to association studies (see the article by Terwilliger and Weiss for a detailed discussion (74)). Association studies of candidate gene loci and disease phenotypes are unlikely to be successful in identifying the genetic factors that influence Chagas' disease. Only family studies can provide detailed quantification of the genetic effects that may influence infection with *T. cruzi* and subsequent disease progression.

5. GENETIC QUESTIONS ABOUT CHAGAS' DISEASE-RELATED PHENOTYPES

Genetic analyses can be informative for a variety of questions concerning the variation in susceptibility of humans to infection with *T. cruzi* and differential disease progression in infected individuals. The first question that presents itself concerns whether or not there is a genetic component to susceptibility to infection with *Trypanosoma cruzi*. Uninfected individuals are found in all populations where *T.* cruzi infections are highly prevalent, despite the probable exposure of these individuals to bites from infected reduviid bugs. If genetic determinants affect

susceptibility to infection, their identification could provide biological pathways to be targeted in the development of new methods for preventing *T. cruzi* infection. As outlined above, complete elimination of infected vectors is unlikely given the sylvatic cycle for the disease, and prevention of infections will continue to be a public health priority especially for populations moving into previously uninhabited areas, such as the Brazilian Amazon, that harbor triatomid bugs.

Among those infected with *Trypanosoma cruzi* there is considerable variation in disease outcome. Understanding the genetic determinants of response to long-term *T. cruzi* infection may contribute to the development of new treatment strategies applicable to the millions already infected with the disease. It would be useful to know if there are host genetic factors that determine whether those experiencing Chagas' disease will develop the cardiac or the gastrointestinal form of the disease. Similarly, genetic epidemiological approaches can be used to assess whether or not there are host genetic factors that determine the severity of the cardiac form of the disease, as reflected in electrocardiographic measures, in individuals with Chagas' disease-related cardiomyopathy.

Quantitative genetic methods are used to determine how much of the variation in a trait is attributable to genetic factors; a measure referred to as the heritability. In a variance components approach, the observed variation in a trait is partitioned into components attributable to additive genetic factors (the heritability), systematic environmental factors (e.g., common household effects), and random environmental factors.

While it is possible to account for systematic environmental factors in a quantitative genetic approach, there is always the potential for confounding between genetics and environment since nuclear families tend to share a common household and hence a common local environment. A genome scan based on extended pedigrees is the ultimate way to discriminate genetic and environmental effects on these traits because it is impossible for correlations due environmental factors to show a pattern consistent with Mendelian segregation throughout an extended pedigree. True genetic effects are characterized through a genome scan, which allows eventual identification of the specific genes determining observed epidemiological patterns of infection and disease progression.

6. FAMILY STUDIES OF SUSCEPTIBILITY TO CHAGAS' DISEASE

Large extended pedigrees provide the most powerful sampling design for genetic epidemiological studies of common diseases, i.e. diseases that affect 10% or more of the population. The power of the pedigree for statistical genetic analyses, including quantitative genetic and linkage analyses, is a function of the size and complexity of the pedigree (75-76).

There has been considerable interest in establishing large-scale genetic epidemiological studies of Chagas' disease (e.g., 60-61), but there are only two published reports which used extended family data to assess the genetic components of *T. cruzi* infection or correlates of Chagas' disease. In one of the reports, the genetic determinants of immunoglobulin levels in a population with endemic Chagas' disease were quantified using a path analysis approach (77). Significant heritabilities were found for both immunoglobulin A and immunoglobulin G in the Brazilian population under study with approximately 33% of the variation in each of these traits, which were proposed to be associated with risk of Chagas' disease, attributable to genetic factors.

The other published genetic epidemiological study, which was based on extended pedigree information, measured the heritability of seropositivity to *T. cruzi* infection in humans (78). The study examined variation in seropositivity assessed for 716 residents of the region of Posse in the state of Goiás, Brazil. Pedigrees were reconstructed using family information collected during house to house surveys, and 525 of the individuals could be assigned to a total of 146 pedigrees ranging in size between 2 and 103 individuals. The remaining 191 individuals were treated as independents, and retained in the analysis to improve estimation of the common household effects. A variance components analysis approach was used to evaluate a series of nested models for the determinants of the observed variation in the seropositivity trait. The qualitative seropositivity trait was treated as a threshold trait having an underlying continuous distribution of liability. The various models allowed for the observed pattern of variation to be random, to be due solely to genetic factors, to be attributable solely to systematic environmental factors as assessed by common household, or to be due to a combination of environmental and genetic factors. The best fitting model for the observed variation in the trait was a model which included both household and genetic effects. The heritability of seropositivity was determined to be 0.556 and was highly significant ($p < 0.005$), indicating that 56% of the observed variation in the seropositivity trait was attributable to genetic effects. A further 23% of the variation was attributable to common household effects.

7. FUTURE OF GENETIC RESEARCH ON CHAGAS' DISEASE

The family study described above was a preliminary quantitative genetic assessment of the influence of genetic factors on variation in seropositivity for *T. cruzi* infection. However, given the inability to fully characterize the known environmental influences on susceptibility, this high heritability is a strong indicator that genetic factors have a role in determining the distribution of seropositive and seronegative people in the studied population. However, a high heritability alone cannot be considered conclusive. Linkage analyses in extended pedigrees are the ultimate way to discriminate genetic effects, since environmental influences cannot segregate throughout an

extended pedigree in a manner consistent with the known Mendelian ratios of genetic effects.

In the past decade, rapid advances have been made in the statistical genetic analysis of quantitative phenotypes (79). Methods have been developed that allow the observed variation in physiological and disease phenotypes to be partitioned among environmental and genetic components. Genetic epidemiological models permit partitioning the environment into random and systematic components (80). Household, socioeconomic, behavioral, or ecological factors leading to differential exposure to parasites can be directly incorporated into the models. For example, household conditions and occupational activities can be included. These new models allow discrimination between hypotheses of differential exposure versus genetic determination given the appropriate data structure.

Linkage-based genomic scanning methodologies now allow us to find and characterize specific loci influencing complex diseases (79, 81). The genomic scan approach involves placing random genetic markers every 10cM throughout the entire genome. Such complete coverage of the genome makes it possible to detect all relevant genes influencing the phenotypes of interest, avoids all the potential problems inherent to a candidate gene approach, and maximizes the likelihood of successfully detecting genetic effects if they exist. Variance components analysis methods, such as those implemented in SOLAR, allow the efficient detection of linkage between the genetic markers and the disease-related qualitative or quantitative phenotypes characterized for the pedigree under study (82).

Despite the fact that genome scanning approaches have only been implemented within the last five years, there have been significant results in a number of diseases. For example, genes have been mapped for non-insulin dependent diabetes, obesity, and alcoholism (83-90). While applications of genomic scanning to infectious diseases are few, the genomic scan approach is likely to lead to new insights in this area as evidenced by the finding of quantitative trait loci influencing susceptibility to schistosomiasis (10) and ascariasis (9).

The identification of the specific genes influencing *T. cruzi* infection and Chagas' disease through linkage analyses of genome scan data from large extended pedigrees will enable identification of the individuals most likely to progress to severe disease, and will allow medical interventions to be targeted to the individuals most in need of care. Ultimately, knowledge of the genes influencing the disease and its correlates will facilitate drug development efforts aimed at the biological pathways identified through genetic analyses (91-93). The current lack of effective preventions or treatments for Chagas' disease makes this an ideal candidate for drug discovery based on the results of genome scan studies.

8. PERSPECTIVE

Chagas' disease remains the leading cause of heart disease in South and Central America despite decades of vector control efforts aimed at eliminating transmission of the disease. With more than 16 million individuals currently infected with *T. cruzi* and the tremendous costs that will be associated with the care of these individuals, it is clear that Chagas' disease will remain a major public health concern in the Americas for the foreseeable future. Genetic approaches can yield new insights into the determinants of infection disease progression. In particular, the identification of the specific genes that influence disease severity will suggest biological pathways to be targeted for drug development efforts. Genome scanning efforts are still in their infancy, and there have been few applications to parasitic diseases. However, the successes already achieved indicate the bright future of genome scans for improving our understanding of the determinants of human disease, and particularly of Chagas' disease.

9. ACKNOWLEDGMENTS

This review was supported by NIH grant HL66480.

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Key Words: Quantitative genetic analysis, genome scan, linkage analysis, human population studies, pedigrees, Review

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