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GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

ACAT-1 gene rs1044925 SNP and its relation with different clinical forms of chronic Chagas disease

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Abstract

Chagas disease, caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), although discovered more than a century ago, is still a not very well-elucidated aspect. Individuals in the chronic phase of the disease may present asymptomatic clinical form or symptomatologies related to the cardiac, digestive systems, or both (mixed clinical form). SNPs (*single nucleotide polymorphisms*) have been identified as important markers because they constitute about 90% of the variation in the human genome. One of them is localized to the *ACAT-1* gene (*cholesterol acyltransferase 1*) (rs1044925) and has been linked to lipid disorders. Some studies have suggested the interaction between *T. cruzi* and the lipid metabolism of the host. Therefore, the objective of the present study was to evaluate the association between the *ACAT-1* gene rs1044925 SNP in relation to clinical manifestations in patients with chronic Chagas disease. A total of 135 individuals with chronic Chagas disease, 86 (63.7%) asymptomatic individuals and 49 (36.3%) symptomatic patients (22 with cardiac clinical form, 18 with digestive form and 9 with mixed form) participated in the study. To evaluate the polymorphism, the PCR-RFLP technique were used. There was a significant difference and a higher frequency of AA and AC genotypes ($p = 0.047$ and $p = 0.016$, respectively) of the *ACAT-1* gene in asymptomatic chagasic individuals. The result suggests a protective character of the AA and AC genotypes of the rs1044925 SNP in relation to the presence of symptomatic clinical manifestations of the disease in chronic chagasic individuals.

Keywords Chagas disease · *Single nucleotide polymorphisms* (SNPs) · ACAT-1 · Molecular markers

Introduction

Chagas disease caused by the protozoan *Trypanosoma cruzi* is still endemic in many countries (Andrade et al. 2014).

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According to the World Health Organization, it is estimated that eight million people around the world are infected and the infection has spread to non-endemic regions (WHO 2013). In the USA, for example, about 300,000 people are infected (CDC 2006). In Brazil, it is estimated that between two and three million people have acquired the disease and 600,000 have symptoms related to the chronic phase (Bello Corassa et al. 2017).

The disease has two clinical phases: acute and chronic. In the acute phase, individuals present high parasitemia and non-specific symptoms such as malaise, fever, hepatosplenomegaly, and atypical lymphocytosis (Bern 2015). After 2 to 4 months, individuals enter the chronic phase of the disease and may present asymptomatic clinical form or symptomatologies related to the cardiac, digestive systems, or both (mixed clinical form) (Dutra et al. 2009).

Approximately 70% of the infected individuals have an undetermined clinical form of the disease (Rassi et al. 2012) and presents no abnormalities in physical and radiological exams or symptoms of the disease (Dias et al. 2016).

The cardiac clinical form, which affects approximately 20% of the infected individuals, corresponds to the most serious manifestation of the disease and may promote arrhythmias, thromboembolism, and heart failure in these patients (Rassi et al. 2009).

On the other hand, the digestive clinical form involves mainly damage to the esophagus and colon with consequent megaesophagus and megacolon. The most common manifestations of this clinical form are as follows: mild achalasia, motility disorders, dysphagia, and regurgitation (Bern 2015).

A small percentage of chronic chagasic patients (2–5%) present clinically for both digestive and cardiac manifestations of the disease and are referred to as mixed or cardiodigestive form (Rassi et al. 2010). One of the most intriguing questions of the disease's chronic phase is the fact that leads individuals to present different clinical manifestations. So far, the main factor responsible for individuals developing and not developing symptoms of the disease is not known. Information on human genetic predisposition has progressed greatly in recent years and the search for host molecular markers are potential targets for explaining susceptibility in some infections (Burgner et al. 2006).

One type of marker widely used in genetic studies comprises the *single nucleotide polymorphisms* (SNPs), which result in an exchange of one nucleotide for another in a DNA sequence (Beuzen et al. 2000). They represent for about 90% of the variation in the human genome (Collins et al. 1998) and have a frequency of more than 1% in the human population (Guerra and Yu 2006). Because they are fairly common, these polymorphisms have been extensively investigated. Some studies have already demonstrated the role of these polymorphisms in susceptibility to some common diseases such as diabetes and cancer (London et al. 1997; Marcus et al. 2000; Altshuler et al. 2000) and progression to severe forms of infectious diseases such as malaria (McGuire et al. 1994) and leprosy (Moraes et al. 2004).

The SNP rs1044925 (A/C) is located on the *ACAT-1* (*cholesterol acyltransferase 1*) gene and has been extensively investigated in lipid disorders. This gene encodes the ACAT-1 enzyme that is responsible for esterification of intracellular cholesterol (Chang et al. 1993) and has been related to the formation of foam cells in macrophages, which is a characteristic of the first stages of atherosclerotic lesions (Li et al. 2006).

With regard to this polymorphism, Wu et al. (2010) found that in a population of China, the AC and CC genotypes of rs1044925 were associated with low levels of total cholesterol and LDL-C in women, when compared to the AA genotype. In another study, the AC and CC genotypes were associated with increased systolic pressure in smokers (Yin et al. 2012). The rs1044925 SNP AC and CC genotypes were also associated with higher levels of total cholesterol, HDL-C, and ApoAI in the serum of men with hypercholesterolemia (Wu et al. 2012).

These studies demonstrate the role of the *ACAT-1* gene rs1044925 SNP and its relationship to some disorders that prone a risk for cardiovascular disease. In addition, some studies have suggested that there is an interaction between *T. cruzi* and the lipid metabolism of the host (Miao and Ndao 2014).

To date, no study has investigated this SNP in individuals with Chagas disease. Therefore, the present study aims to evaluate the role of the rs1044925 SNP of the *ACAT-1* gene in patients with and without clinical manifestations of the disease.

Material and methods

Study subjects

A total of 135 individuals that attended at the Tropical Diseases Outpatient Clinic of Botucatu Medical School (UNESP), in the interior of the State of São Paulo, Brazil, were evaluated. Botucatu is located between 22° 53′ 09″ to the south and 48° 26′ 42″ to the west.

The included individuals were of both sexes and ranged in age from 36 to 83 years, presenting different clinical forms of chronic Chagas disease. In order to confirm the diagnosis of Chagas disease, it was verified positivity for *T. cruzi* serological tests using the Chemiluminescence, Indirect Immunofluorescence, and Hemagglutination assay.

Subjects with undetermined clinical form were classified by the absence of symptoms of the disease and normal results in conventional electrocardiogram examinations, radiological study of the thorax, esophagus, and colon.

Individuals with cardiac clinical form were classified by electrocardiogram abnormalities (mainly non-specific alterations of the ventricular repolarization followed, left atrial overload, prolonged QTc interval, low QRS complex voltage, atrial fibrillation, atrioventricular block, branch block, and sinus tachycardia) (Souza et al. 2013). Changes in echocardiogram examinations may also be observed, such as the presence of intracavitary thrombi, pericardial effusion, enlargement of the cardiac chambers, atrioventricular valve regurgitation, and impairment of left ventricular systolic function (Dias et al. 2016).

The classification of the digestive form of the disease mainly involves changes in the esophagus and colon. The results of chest X-ray in PA or profile (to check the presence of achalasia), contrasting the esophagus radiography, radiography of the abdomen, and contrast radiography of the colon-barium enema examinations were evaluated (Dias et al. 2016).

Subjects with a mixed (cardiodigestive) form of the disease were classified due to the presence of changes in the cardiac and digestive clinical form of the disease, concomitantly.

The study subjects were divided into two groups: group 1 consisting of chronic Chagas individuals with an asymptomatic form of Chagas disease and group 2 consisting of chronic chagasic symptomatic individuals with clinical, cardiac, digestive, and mixed forms of the disease.

The results of the exams were verified by consulting the patients' medical records and, from this, information was collected from individuals: naturalness, origin, age, weight and height (in order to calculate body mass index), and comorbidities for characterization of the sample.

Exclusion criteria

From this study, pregnant women were excluded.

Ethics committee

All individuals accepted to participate in the study by signing the Informed Consent Term applied at the time of the consultation. The study was approved by the Research Ethics Committee of the Faculty of Medicine of Botucatu—UNESP (CEP—Opinion no. 1,576,662).

Calculation of body mass index

In relation to anthropometric data, the weight in kilograms (kg) and height in centimeters (cm) were considered. The following formula was used to calculate the body mass index (BMI): weight / height × height.

The classification for adults (20 to 59 years) was applied using the following values: < 18.5 were classified as low weight; ≥ 18.5 and < 25 eutrophy; ≥ 25 and < 30 were overweight and ≥ 30 were classified as obese. For the elderly (> 60 years), the classification of the values was ≤ 22 for those with low weight, > 22 and < 27 eutrophic, and ≥ 27 overweight (Ministério da Saúde 2004).

Blood collection and leukocytes isolation

Five milliliters of peripheral blood was collected from the study subjects, and after centrifugation of 15 min at 15,000 rpm, the separation of the WBC was performed. In this portion of the blood was added 1 mL of red cell lysis buffer (RCLB (pH: 7.6 1X: 10 mM Tris; 5 mM $MgCl_2$ and 10 mM NaCl) and centrifugation performed at 15,000 rpm for 10 min. The supernatant was discarded and the pellet stored at − 20 °C.

DNA extraction

DNA extraction was performed with the PureLink® Genomic DNA Mini Kits K182001 (Invitrogen, Carlsbad, USA) according to the manufacturer's recommendations and the DNA was quantified in NanoVue Plus spectrophotometer apparatus (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Polymerase chain reaction

Forward primers 5′-TATATTAAGGGGATCAGAAGT-3′ and reverse 5′-CCACCTAAAAACATACTACC-3′ were used for the *ACAT-1* gene.

Polymerase chain reaction (PCR) was performed in a final volume of 25 μl containing 2.5 μl of genomic DNA, 10 pmol of each primer, 1.0 unit of Taq polymerase, and 1.0 mM of $MgCl_2$, 500 μM of dNTPs. Cycles were performed according to Wu et al. (2010) with some modifications. Initial denaturation at 95 °C for 5 min, 35 cycles with denaturation at 95 °C for 45 s, annealing at 55 °C for 1 min, extension at 72 °C for 50 s, and final cycle at 72 °C for 10 min. Verification of the amplified product was performed on 2% agarose gel to observe the 389 bp fragment.

Restriction enzyme digestion

For analysis of the *ACAT-1* gene SNP (rs1044925), 10 μL of the amplified PCR product and 0.5 μL of restriction enzyme RsaI-Rhodopseudomonas sphaeroides (New England BioLabs, Boston, MA, USA-R0167S) were used for digestion at 37 °C overnight. The results of the digested samples were checked on 2% agarose gel and visualized in ultraviolet transilluminator. The size of the bands for each genotype is as follows: AA—389 bp; AC—389, 279, and 110 bp; and CC—279 and 110 bp (Table 1).

Statistical analysis

For the analysis of the age group of the participants, the mean and standard deviation and Student's *t* test for independent samples were performed (Zar 1999). Comparison between general patient data, genotypes, and allelic frequencies was performed using the Goodman association test, supplemented with multiple comparisons between and within multinomial

Table 1 Relation of genes and as respective restriction enzymes, enzyme cleavage site, and genotypes surveyed in patients with chronic symptomatic and asymptomatic Chagas disease

Gene/SNP	Restriction enzyme	Enzyme cutting site	Genotypes
ACAT-1 (rs1044925)	RsaI	5′...GT↓AC...3′3′...CA↑TG...5′	AA—389 bp; AC—389, 279 e 110 bp; CC—279 e 110 bp

populations (Goodman 1964, 1965), considering the 5% level of significance. Allelic frequencies were tested for Hardy-Weinberg equilibrium.

Results

Sample characterization

The 135 chagasic patients came mainly from the interior of São Paulo State (SP), Brazil. Only two individuals were from Siqueira Campos, Paraná. Patients were grouped by microregions of São Paulo State, except for two individuals previously mentioned, from Paraná State. The microregions, in particular, correspond to the patient's origins, mainly: Itapeva (45), Botucatu (33), Avaré (31), and Ourinhos (13) (Fig. 1). All individuals are Brazilian and natural from cities of São Paulo, Minas Gerais, Paraná, Pernambuco, Bahia, Alagoas, and Mato Grosso States. Among them, 18 patients were from Itaporanga (SP), 14 from Itaí (SP), and 9 from Taquarituba (SP) (Fig. 2).

Of the 135 chagasic patients included in the study, 86 (63.7%) were asymptomatic and 49 (36.3%) were symptomatic. In the group of symptomatic individuals, 22 (44.9%) presented cardiac form of the disease, 18 (36.7%) individuals presented digestive form, and nine (18.4%) individuals had mixed form of the disease.

Among the asymptomatic patients, 46 (53.5%) were men and 40 (46.5%) were women; in the symptomatic group, 23 (46.9%) were men and 26 (53.1%) women. The mean age in the group of asymptomatic individuals was 59.0 ± 8.0 years (mean ± standard deviation) and in the symptomatic group, 61.5 ± 9.1 years. There was no significant difference between sex and mean age between groups ($p > 0.05$).

In relation to the body mass index, 48.8% of the asymptomatic individuals and 36.7% of the symptomatic individuals were overweight.

Of the 135 patients included in our study, 98 (72.6%) had comorbidities such as hypertension, hypercholesterolemia, diabetes, or association between them.

Among the asymptomatic individuals, 57 (58.2%) had comorbidities. Fifteen patients (26.3%) had hypercholesterolemia and 24 (42.1%) had an association of more than one comorbidity. Among the symptomatic individuals, 41 (41.8%) individuals had comorbidities. Eleven (26.9%) were hypertensive and 24 (58.5%) had comorbidity associations (Table 2). There was no significant difference when comparing all variables of "BMI" and "comorbidities" between the groups ($p > 0.05$).

ACAT-1 gene rs1044925 SNP genotyping

For the rs1044925 SNP genotypes, the samples were presented in Hardy-Weinberg equilibrium. Regarding the results of the polymorphism genotypes of *ACAT-1* gene among the groups, there was a higher frequency of the genotypes AA and AC in asymptomatic individuals compared with symptomatic individuals ($p = 0.047$ and $p = 0.016$). There was no significant difference between the CC genotype when compared between the two groups ($p > 0.05$). There was also no

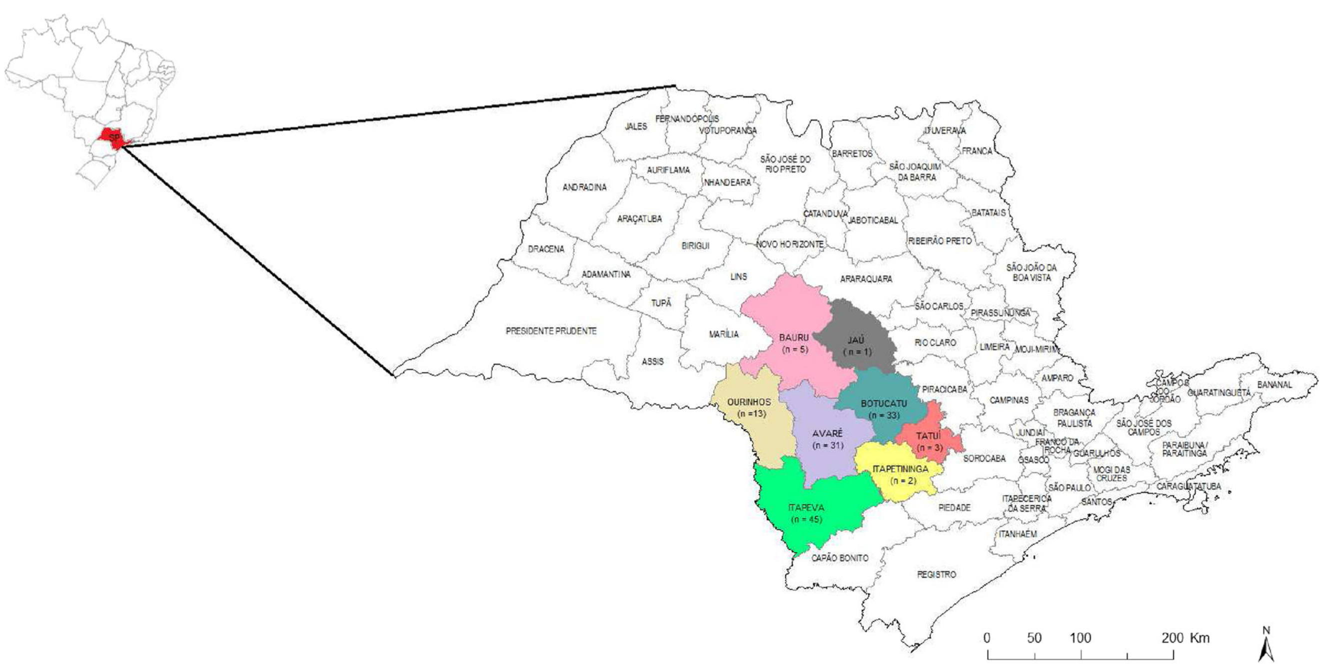

Fig. 1 Origin of chronic chagasic patients grouped by microregions of the State of São Paulo, Brazil, genotyped for *ACAT-1* gene*, *Except for two patients from Siqueira Campos, Praná, Brazil

State	City	n	State	City	n
São Paulo	Itaporanga	18	Minas Gerais	Cristalia	2
	Itaí	14		Berilo	1
	Taquarituba	9		Botumirim	1
	Taguaí	7		Capelinha	1
	Barão de Antonina	5		Coromandel	1
	Coronel Macedo	5		Itabirá	1
	Fartura	4	Paraná	Ribeirão do Pinhal	3
	Paranapanema	4		Joaquim Tavora	2
	Angatuba	3		Santo Antonio da Platina	2
	Itaberá	3		Andirá	1
	Botumirim	2		Carlópolis	1
	Iaras	2		Conselheiro Mairink	1
	Itatinga	2		Ibaiti	1
	Aguas de Sta Barbara	1		Jacarezinho	1
	Arandu	1		Araruva	1
	Avaré	1		Ribeirão Claro	1
	Bernardino de Campos	1		Salto de Lontra	1
	Bofete	1		Santana do Itararé	1
	Borebi	1		Siqueira Campos	1
	Botucatu	1		Tomazina	1
	Cerqueira César	1		Wenceslau Braz	1
	Guariba	1	Pernambuco	Carpina	1
	Ibirá	1		Itapetim	1
	Ibirarema	1	Bahia	Castro Alves	1
	Itapetininga	1		Ituaçu	1
	Pardinho	1		Miguel Calmon	1
	Piraju	1		Pindaí	1
	Platina	1		Urandi	1
	Porangaba	1	Alagoas	Marimbondo	1
	Pratânia	1		Pindoba	1
	São Pedro do Turvo	1	Mato Grosso	Poxoréu	1
	São Roque	1			
	Sarutaia	1			
	Tanabi	1			

Fig 2 Relation of the place of birth of chronic chagasic patients genotyped for the *ACAT-1* gene according to the Brazilian States

significant difference between the AA, AC, and CC genotypes when compared within the groups ($p > 0.05$) (between asymptomatic individuals and between symptomatic individuals) (Table 3). We evaluated the following parameters associated with cholesterol metabolism: total cholesterol, HDL, and triglycerides. Of these, only the relationship between the presence of the A allele with high cholesterol levels was statistically significant using the ANOVA test ($p < 0.05$).

Table 2 Distribution of sex, mean age and standard deviation, body mass index, and comorbidities of 135 chronic chagasic patients subdivided by asymptomatic and symptomatic clinical form, from different Brazilian municipalities

Clinical forms ($n = 135$)			
	Asymptomatic ($n = 86$)	Symptomatic ($n = 49$)	p value
Sex n (%)			
Male	46 (53.5)	23 (46.9)	$p > 0.05$
Female	40 (46.5)	26 (53.1)	$p > 0.05$
Total n (%)	86 (100)	49 (100)	
Mean age and standard deviation	59.0 ± 8.0 years	61.5 ± 9.1 years	$p > 0.05$
Body mass index n (%)			
Underweight	3 (3.5)	4 (8.2)	$p > 0.05$
Normal range	27 (31.4)	17 (34.7)	$p > 0.05$
Overweight	42 (48.8)	18 (36.7)	$p > 0.05$
Obese	14 (16.3)	10 (20.4)	$p > 0.05$
Total n (%)	86 (100)	49 (100)	
Comorbidities n (%)			
Hypertension	12 (21.1)	11 (26.9)	$p > 0.05$
Hypercholesterolemia	15 (26.3)	5 (12.2)	$p > 0.05$
Diabetes	6 (10.5)	1 (2.4)	$p > 0.05$
Associated comorbidities	24 (42.1)	24 (58.5)	$p > 0.05$
Total	57 (58.2)	41 (41.8)	

Goodman's association test considering the level of significance of 5%. p value: comparison of response categories (columns) within the group (rows). The Student's t test was applied for independent samples

Table 3 Distribution of *ACAT-1* genotypes according to asymptomatic and symptomatic patients with chronic Chagas disease, coming from different Brazilian municipalities

ACAT-1 genotypes	Clinical forms *n* (%)		
	Asymptomatic	Symptomatic	Total
AA	34 (63.0) B[a]	20 (37.0) A[a]	54 (40.0)
AC	43 (66.2) B[a]	22 (33.8) A[a]	65 (48.1)
CC	9 (56.3)A[a]	7 (43.7) A[a]	16 (11.9)
TOTAL	86 (63.7)	49 (36.3)	135 (100)

Goodman's association test considering the level of significance of 5%. Capital letters: comparing response categories (columns) within the group (rows), where B > A and AB does not differ from A and B. Lower case letters: group comparison (rows) fixed to response category (column), being b > a and ab does not differ from a and b

Regarding the allelic frequencies, there was no significant difference between the A and C alleles when compared to and within the groups of asymptomatic and symptomatic individuals ($p > 0.05$) (Table 4). The results were shown as AA, AC, and CC genotypes in agarose gel photo (Fig. 3).

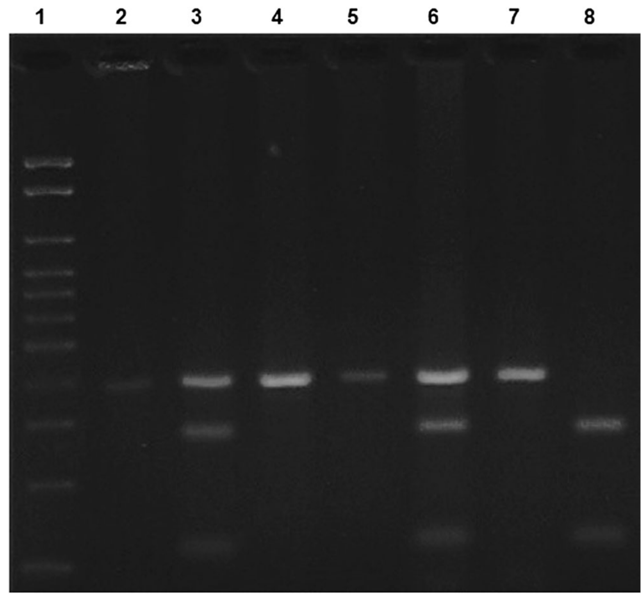

Fig 3 Genotyping of rs1044925 polymorphism in the *ACAT-1* gene. Line 1: 100 bp markee ladder: Lines 2, 4, 5, and 7; AA genotype (389 bp); Lines 3 and 6: AC genotype (389, 279, and 110 bp); Line 8: CC genotype (279 and 110 bp)

Discussion

Chagas disease still raises debate, mainly regarding the different clinical manifestations that chagasic individuals exhibit in the chronic phase. It is believed that factors such as environment, parasite, and genetic factors of the host can contribute to the severity of the disease (Menezes et al. 2016).

In the present study, chronic chagasic patients came mainly from microregions of São Paulo State, such as Itapeva, Botucatu, Avaré, and Ourinhos (Fig. 1). These individuals are native from several states in Brazil, mainly in the interior of São Paulo State, such as Itaporanga, Itaí, and Taquarituba (Fig. 2). These regions correspond to the flow of care from the Ambulatory of Tropical Diseases in Botucatu and are inserted in an area endemic for Chagas disease.

Table 4 Distribution of allelic frequencies of the *ACAT-1* gene according to asymptomatic and symptomatic patients with chronic Chagas disease, coming from different Brazilian municipalities

ACAT-1 alleles	Clinical forms n (%)	
	Asymptomatic	Symptomatic
A	97 (56.1) A[a]	76 (43.9) A[a]
C	55 (56.7) A[a]	42 (43.3) A[a]
TOTAL	152	118

Goodman's association test considering the level of significance of 5%. Capital letters: comparing response categories (columns) within the group (rows), where B > A and AB does not differ from A and B. Lower case letters: group comparison (rows) fixed to response category (column), being b > a and ab does not differ from a and b

The mean age in the group of asymptomatic individuals was 59 years and in the symptomatic group, 61.5 years, with no significant difference between these groups. The mean age of Chagas chronic patients suggests the role of the efficacy of Chagas disease vector control programs in the country, which began in the mid-1950s (Pereira and Navarro 2013). However, this factor does not rule out the occurrence of vectors contaminated by *T. cruzi*, since these campaigns were mainly aimed at combating the vector Triatoma infestans (Fitarelli and Horn 2009; Moraes-Souza and Ferreira-Silva 2011; Abad-Franch et al. 2013). In São Paulo State, there are other secondary species of triatomines with potential for infectivity, such as *Panstrongylus megistus* and *Triatoma sordida* (SUCEN - Superintendência de Controle de Endemias 2017).

In addition, as individuals age, there is greater susceptibility to comorbidities, independent of infection with Chagas disease (Alves et al. 2009). In the present study, 66.3% of the asymptomatic chagasic patients presented some comorbidity associated with the infection. The same was observed in 83.7% of symptomatic chagasic patients. Of the total of 135 chagasic patients, 24 (58.5%) of the symptomatic and 24 (42.1%) asymptomatic had an association of more than one comorbidity, in addition to *T. cruzi* infection. It is believed that about 88% of the world's population aged 65 years and over present at least one chronic disorder (Wolff et al. 2002). Individuals who present more than one comorbidity are more prone to physical inactivity and greater occurrence of adverse drug interactions due to polypharmacy (Wolff et al. 2002).

In the group of asymptomatic individuals, 21.1% had hypertension and 26.3% had hypercholesterolemia. In the group of symptomatic individuals, 26.9% were hypertensive and 12.2% had hypercholesterolemia. In a study conducted by Vicco et al. (2014), a higher frequency of hypertension was found in individuals with Chagas disease (34%) when compared to individuals without the infection (13%). Hypertension was also associated with heart failure in chagasic patients (Vicco et al. 2014). In another study, of 878 chagasic patients, 37% had arterial hypertension, mainly individuals with cardiac involvement and > 45 years of age (Guariento et al. 1998). Gurgel and Almeida (2007) analyzed the records of individuals submitted to necropsy and found that of 101 chagasic patients, 33 (32.7%) were hypertensive.

In the present study, 17% of chagasic patients (regardless of presence / absence of clinical symptoms) had hypertension. This portion is lower when compared to the studies cited above, which may be justified by the fact that this disorder is influenced by several factors such as obesity, alcoholism, sex, and age, among others (Gurgel and Almeida 2007). Moreover, the prevalence of hypertension may vary among populations, although the factors that influence these discrepancies are not yet known (Lacruz et al. 2015). However, we observed that this data is similar when compared to the general population in the country in which it is estimated that there are 20% of hypertensive individuals (Passos et al. 2006).

Regarding diabetes, it was found in 5.2% of 135 chagasic patients of the present study. Santos et al. (1999) observed that among 362 chagasic women, there was a prevalence of 10.5% of diabetes mellitus.

The comorbidities found in the present study (hypertension, hypercholesterolemia, and diabetes) are considered risk factors for the development of cardiac conditions such as myocardial infarction (Boysen et al. 1988) and atherosclerosis (Kannel and Sytkowski 1987). In addition to the disorders caused by chronic chagasic cardiomyopathy, this clinical form may increase the risk of infarction in these individuals (Malik et al. 2015).

However, the impact of these morbidities on disease progression is uncertain. The chronic disorders of these elderly individuals, chronic chagasic infection, and their severe clinical forms emphasize the importance of special attention in these patients.

Other information raised in the present study is about BMI. Although no significant difference was found between the BMI and the studied groups, a prevalence of 32.6% of chagasic patients with eutrophy was observed, 44.4% were overweight, and 17.8% were obese in the group of asymptomatic and symptomatic individuals. Geraix et al. (2007) found a prevalence of 62.1% obese in chagasic patients.

According to the Ministry of Health, it is estimated that there are 60% of obese people in the country (Ministério da Saúde 2017). The BMI of individuals may vary according to the study population since they involve environmental, psychosocial, and physiological factors (Kopelman 2000). As with the previously mentioned morbidities, overweight and obesity are considered risk factors for the development of cardiovascular diseases as well as increased blood pressure, cholesterol, and glucose (Poirier et al. 2006; Van Gaal et al. 2006).

In the present study, a significant difference was observed between the AA and AC genotypes of the *ACAT-1* gene rs1044925 SNP among asymptomatic and symptomatic chagasic individuals. This data suggests a protective character in the pathogenesis of the disease among the carriers of these genotypes.

There is no data regarding the SNP allele frequencies in the Brazilian population, specifically. However, according to the 1000 Genomes Database (https://www.ncbi.nlm.nih.gov/snp/rs1044925#frequency_tab), the global frequencies for the alleles are C = 0.267 and A = 0.733. For the American population, the frequencies are C = 0.28 and A = 0.72.

Accordingly, in a study carried out with a population of China, the AC and CC genotypes of rs1044925 were associated with low levels of total cholesterol, LDL-C, and ApoB in women when compared to the AA genotype (Wu et al. 2010). AC and CC genotypes were also associated with increased systolic pressure in smokers (Yin et al. 2012). In another study, the AC and CC genotypes of the rs1044925 SNP were associated with higher levels of total cholesterol, HDL-C, and ApoAI in the serum of men with hypercholesterolemia (Wu et al. 2012).

In the present study, 14.9% of chagasic patients with hypercholesterolemia were observed in both groups. In the literature, only a clinical data survey was found in which the prevalence of hypercholesterolemia was observed in chronic Chagas patients treated in the same outpatient clinic of the present study, in which 22.7% of hypercholesterolemia was found among 66 chagasic patients studied (Geraix et al. 2007).

Cholesterol is an important molecule, constituent of cell membranes that are involved in several physiological processes, such as maintenance of membrane fluidity, bile acid metabolism, and steroid hormones (Rudel and Shelness 2000; Ikonen 2008). The excess of free cholesterol can be harmful to the cells, so it is necessary to convert them into cholesterol esters that are organized into drops of cytoplasmic lipids (Chang et al., 1993; Rogers et al. 2015). It is known that these lipid droplets are involved in several inflammatory processes (Weller et al. 1999; Bandeira-Melo et al. 2002).

Recent studies have evaluated the role of host lipid metabolism in *T. cruzi* infection (Miao and Ndao 2014). Johndrow et al. (2014) found increased levels of intracellular cholesterol in heart tissues of a late stage individual with the cardiac form of Chagas disease, a fact that was not observed in heart tissue of an individual who died due to ischemic heart disease. They also found increased LDL in the pancreas and heart of animals infected with a chronic *T. cruzi* strain 90 days post infection. Fernandes et al. (2007) also found that cholesterol depletion in *T. cruzi* infected cells and infecting forms (trypomastigote) and non-infective (amastigotes) in vitro resulted in the decreased invasion of these parasites, which was recovered after cell cholesterol plenum.

On the other hand, Nagajyothi et al. (2014) observed that mice infected with *T. cruzi* (acute phase strain) and submitted to a high-fat diet showed decreased parasitemia and increased survival rate compared to mice with regular fat diet. It was also observed that the morphological alterations of the heart of mice submitted to rich diet and fat were less pronounced in comparison with the animals that maintained a regular diet of fat.

These studies suggest cholesterol involvement in *T. cruzi* infection. However, much of the literature refers to the acute phase of infection. Differences are also observed regarding the role of cholesterol in the pathogenesis of Chagas disease. In the present study, we evaluated total cholesterol, HDL, and triglycerides. We observed a relationship between the presence of the A allele with high cholesterol levels. Individuals homozygous for A (AA) or heterozygous for A (AC) apparently have higher levels of triglycerides, but not significant. We believe that the presence of Adenine in SNP rs1044925 is associated with elevated cholesterol and this elevation may be associated with the increase in triglycerides.

Conclusion

In the present work, we found a higher frequency between AA and AC genotypes in asymptomatic chagasic individuals when compared to symptomatic individuals. This result suggests that the carriers of these genotypes have a protective character for the development of clinical manifestations of the disease in the chronic phase. This data is unprecedented and can bring great contributions to a better understanding of the pathogenesis of the disease and clinical follow-up of the patients. Further studies should be carried out in order to evaluate the importance of lipid metabolism in the infection and to clarify the functional role of this polymorphism in the clinical manifestation of chagasic individuals in different populations.

Compliance with ethical standards All individuals accepted to participate in the study by signing the Informed Consent Term applied at the time of the consultation. The study was approved by the Research Ethics Committee of the Faculty of Medicine of Botucatu—UNESP (CEP—Opinion no. 1,576,662).

Conflict of interest The authors declare that they have no conflict of interest.

References

Abad-Franch F, Diotaiuti L, Gurgel-Gonçalves R, Gürtler RE (2013) Certifying the interruption of Chagas disease transmission by native vectors: cui bono? Mem Inst Oswaldo Cruz 108:251–254

Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES (2000) The comom PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26:76–80

Alves RM, Thomaz RP, Almeida EA, Wanderley Jda S, Guariento ME (2009) Chagas' disease and ageing: the coexistence of other chronic diseases with Chagas' disease in elderly patients. Rev Soc Bras Med Trop 42:622–628

Andrade DV, Gollob KJ, Dutra WO (2014) Acute chagas disease: new global challenges for an old neglected disease. PLoS Negl Trop Dis 31:e3010

Bandeira-Melo C, Bozza PT, Weller PF (2002) The cellular biology of eosinophil eicosanoid formation and function. J Allergy Clin Immunol 109:393–400

Bello Corassa R, Aceijas C, Alves PAB, Garelick H (2017) Evolution of Chagas' disease in Brazil. Epidemiological perspective and challenges for the future: a critical review. Perspect Public Health 137: 289–295

Bern C (2015) Chagas' disease. N Engl J Med 30:456–466

Beuzen ND, Stear MJ, Chang KC (2000) Molecular markers and their use in animal breeding. Vet J 160:42–52

Boysen G, Nyboe J, Appleyard M, Sorensen PS, Boas J, Somnier F et al (1988) Stroke incidence and risk factors for stroke in Copenhagen, Denmark. Stroke 19:1345–1353

Burgner D, Jamieson SE, Blackwell JM (2006) Genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? Lancet Infect Dis 6:653–663

Center for Disease Control and Prevention (2006) Trypanosomiasis (also known as Chagas disease): Chagas disease. https://www.cdc.gov/parasites/chagas/index.html. Acessed 28 Oct 2017

Chang CC, Huh HY, Cadigan KM, Chang TY (1993) Molecular cloning and functional expression of human acyl-coenzime A: cholesterol acyltransferase cDNA in mutante Chinese hamster ovary cells. J Biol Chem 268:20747–20755

Collins FS, Brooks LD, Chakravarti A (1998) A DNA polymorphism discovery resource for research on human genetic variation. Genome Res 8:1229–1231

Dias JC, Ramos AN Jr, Gontijo ED, Luquetti A, Shikanai-Yasuda MA, Jr C et al (2016) Brazilian consensus on Chagas disease. Epidemiol Serv Saúde 25:7–86

Dutra WO, Menezes CAS, Villani FNA, Costa GC, Silveira ABM et al (2009) Cellular and genetic mechanisms involved in the generation of protective and pathogenic immune responses in human Chagas disease. Mem Inst Oswaldo Cruz 104:208–218

Fernandes MC, Cortez M, Geraldo Yoneyama KA, Straus AH, Yoshida N, Mortara RA (2007) Novel strategy in *Trypanosoma cruzi* cell invasion: implication of cholesterol and host cell microdomains. Int J Parasitol 37:1431–1441

Fitarelli DB, Horn JF (2009) Disposal of blood units due to reactivity for Chagas' disease in a blood donor serological screening laboratory in Porto Alegre, Brazil. Rev Bras Hematol Hemoter 31:310–314

Geraix J, Ardisson LP, Marcondes-Machado J, Pereira PC (2007) Clinical and nutricional profile of individuals with Chagas disease. Braz J Infect Dis 11:411–414

Goodman LA (1964) Simultaneous confidence intervals for constrasts among multinomial populations. Ann Math Stat 35:716–725

Goodman LA (1965) On simultaneous confidence intervals for multinomial proportions. Technometrics 7:247–254

Guariento ME, Orosz JEB, Gontijo JAR (1998) Interação clínica entre moléstia de Chagas e hipertensão arterial primária em um serviço de referência ambulatorial. Arq Bras Cardiol 70:431–434

Guerra R, Yu Z (2006) Single nucleotide polymorphisms and their applications. In: Zhang W, Shmulevich I (eds) Computational and statistical approaches to genomics. Springer, Boston, pp 311–349

Gurgel CBFM, Almeida EA (2007) Frequência da hipertensão arterial em chagásicos crônicos e sua repercussão no coração: estudo clínico e anatomopatológico. Arq Bras Cardiol 89:191–200

Ikonen E (2008) Cellular cholesterol trafficking and compartmentalization. Nat Ver Mol Cell Biol 9:125–138

Johndrow C, Nelson R, Tanowitz H, Weiss LM, Nagajyothi F (2014) *Trypanosoma cruzi* infection results in an increased in intracelullar cholesterol. Microbes Infect 16:337–344

Kannel WB, Sytkowski PA (1987) Atherosclerosis risk factors. Pharmacol Ther 32:207–235

Kopelman PG (2000) Obesity as a medical problema. Nature 404:635–643

Lacruz ME, Klutting A, Hartwig S, Löer M, Tiller D, Greiser KH et al (2015) Prevalence and incidence of hypertension in the general adult population: results of the CARLA-cohort study. Medicine (Baltimore) 94:e952

Li BL, Chang TY, Chen J, Chang CC, Zhao XN (2006) Human ACAT1 gene expression and its involvement in the development of atherosclerosis. Futur Cardiol 2:93–99

London SJ, Lehman TA, Taylor JÁ (1997) Myeloperoxidase genetic polymorphisms and lung câncer risk. Cancer Res 57:5001–5003

Malik LH, Singh GD, Amsterdam EA (2015) The epidemiology, clinical manifestations, and management of Chagas heart disease. Clin Cardiol 38:565–569

Marcus PM, Hayes RB, Vineis P, Garcia-Closas M, Caporaso NE, Autrup H et al (2000) Cigarrette smoking, N-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. Cancer Epidemiol Biomark Prev 9:461–467

McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D (1994) Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malária. Nature 371:508–510

Menezes CAS, Chaves AT, Rocha MOC (2016) Pathogenicity of Chagas disease cardiopathy. JSM Atheroscler 1:1009

Miao Q, Ndao M (2014) *Trypanosoma cruzi* infection and host lipid metabolismo. Mediat Inflamm 902038

Ministério da saúde (2004) Sistema de Vigilância Alimentar e Nutricional – Notas técnicas. http://tabnet.datasus.gov.br/cgi/SISVAN/CNV/notas_sisvan.html. Acessed 11 Jan 2018

Moraes MO, Pacheco AG, Schonkeren JJ, Vanderborght PR, Nery JA, Santos AR, Moraes ME, Moraes JR, Ottenhoff THM, Sampaio EP, Huizinga TWJ, Sarno EN (2004) Interleukin-10 promoter single nucleotide polymorphisms as markers for disease susceptibility and disease severity in leprosy. Genes Immun 5:592–595

Moraes-Souza H, Ferreira-Silva MM (2011) Control of transfusional transmission. Rev Soc Bras Med Trop 44:64–67

Nagajyothi F, Weiss LM, Zhao D, Koba W, Jelicks LA, Cuj MH et al (2014) High fat diet modulates *Trypanosoma cruzi* infection associated myocarditis. PLoS Negl Trop Dis 8:e3118

Passos VMA, Assis TD, Barreto SM (2006) Hipertensão arterial no Brasil: estimativa de prevalência a partir de estudos de base populacional. Epidemiol Serv Saúde 15:35–45

Pereira PC, Navarro EC (2013) Challenges and perspectives of Chagas disease: a review. J Venom Anim Toxins Incl Trop Dis 19:34

Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH, American Heart Association, Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity and Metabolism. Circulation 113:898–918

Portal da Saúde - Ministério da Saúde. Brasília (2017) http://u.saude.gov.br/index.php/o-ministerio/principal/secretarias/sas/sas-noticias/28110-em-dez-anos-obesidade-cresce-60-no-brasil-e-colabora-para-maior-prevalencia-de-hipertensao-e-diabetes. Acessed 11 Jan 2018

Rassi A Jr, Rassi A, Marin-Neto JÁ (2009) Chagas heart disease: pathophysiologic mechanisms, prognostic factors and risk stratification. Mem Inst Oswaldo Cruz 104:152–158

Rassi A Jr, Rassi A, Marin-Neto JÁ (2010) Chagas disease. Lancet 375:1388–1402

Rassi A Jr, Rassi A, Marcondes de Rezende J (2012) American trypanosomiasis (Chagas disease). Infect Dis Clin N Am 26:275–291

Rogers MA, Liu J, Song BL, Li BL, Chang CC, Chang TY (2015) Acyl-CoA: cholesterol acyltransferases (ACATs/SOATs): enzymes with multiple sterols as substrate and as activators. J Steroid Biochem Mol Biol 151:102–107

Rudel LL, Shelness GS (2000) Cholesterol esters and atherosclerosis-a game of ACAT and mouse. Nat Med 6:1313–1314

Santos VM, Cunha SFC, Teixeira VPA, Monteiro JP, Santos JAM, Santos TAM et al (1999) Frequência de *diabetes mellitus* e hiperglicemia em mulheres chagásicas e não-chagásicas. Rev Soc Bras Med Trop 32:489–496

Souza DSM, Almeida AJB, Costa FA, Costa EG, Figueiredo MTS, Póvoa RMS (2013) Eletrocardiograms in the acute phase of Chagas disease throught oral transmission. Rev Bras Cardiol 26:127–130

SUCEN – Superintendência de Controle de Endemias: Situação atual – Vigilância entomológica dos vetores. http://www.saude.sp.gov.br/sucen-superintendencia-de-controle-de-endemias/programas/doenca-de-chagas/situacao-atual. Acessed 4 Dec 2017

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444:875–880

Vicco MH, Rodeles L, Yódice A, Marcipar I (2014) Chagas disease, a risk factor for high blood pressure. Blood Press 23:345–348

Weller PF, Bozza PR, Yu W, Dvorak AM (1999) Cytoplasmatic lipid bodies in eosinophils:central roles in eicosanoid generation. Int Arch Allergy Immunol 118:450–452

Wolff JL, Starfield B, Anderson G (2002) Prevalence, expenditures and complications of multiple chronic conditions in the elderly. Arch Intern Med 162:2269–2276

World Health Organization (2013) Chagas disease (American trypanosomiasis). http://www.who.int/mediacentre/factsheets/fs340/en/. Acessed 28 Oct 2017

Wu DF, Yin RX, Aung LH, Hu XJ, Cao XL, Miao L, Li Q, Yan TT, Wu JZ, Pan SL (2010) Polymorphism of rs1044925 in the acyl-CoA: cholesterol acyltransferase-1 gene and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. Lipids Health Dis 9:139

Wu DF, Yin RX, Aung LH, Li Q, Yan TT, Zeng XN, Huang KK, Huang P, Wu JZ, Pan SL (2012) Sex-specific association of ACAT-1 rs1044925 SNP and serum lipid levels in the hypercholesterolemic subjects. Lipids Health Dis 11:9.7

Yin RX, Wu DF, Wu JZ, Cao XL, Aung LH, Miao L, et al (2012) Interactions of several lipid-related gene polymorphisms and cigarette smoking on blood pressure levels 8:685–696

Zar JH (1999) Bioestatistical analysis, 4th edn. Prentice Hall, New Jersey