BAT1, a Putative Anti-Inflammatory Gene, Is Associated with Chronic Chagas Cardiomyopathy

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Background. It is not understood why only a subset of individuals infected with Trypanosoma cruzi develop chronic Chagas cardiomyopathy (CCC). Patients with CCC display high levels of circulating proinflammatory cytokines. Heart-infiltrating lymphocytes from patients with CCC also express proinflammatory cytokines (tumor necrosis factor–α and interferon-γ) that are detectable in biopsy samples and surgical heart-tissue samples. BAT1, a putative anti-inflammatory gene, presents functional polymorphisms in its promoter region that influence its transcriptional level.

Methods. We assessed, by polymerase chain reaction restriction fragment–length polymorphism analysis, BAT1 variants in the promoter region at positions −22C/G and −348C/T in 154 patients with CCC and in 76 T. cruzi–infected but asymptomatic (ASY) patients.

Results. Of the patients with CCC, 16% were homozygous for the −22C allele, compared with 4% of the ASY patients (P = .004; odds ratio [OR], 4.7 [95% confidence interval {CI}, 1.4–16]). A similar trend was observed for the −348C homozygotes (P = .01; OR, 1.9 [95% CI, 1.0–3.5]). Susceptibility to CCC was conferred by the C variants at nt −22 (P = .003; OR, 1.8 [95% CI, 1.2–2.8]) and at nt −348 (P = .02; OR, 1.7 [95% CI, 1.0–2.8]).

Conclusions. BAT1 variants previously associated with reduced expression of HLA-B–associated transcript 1 are predictive of the development of CCC. These variants may be less efficient in down-regulating inflammatory responses and may contribute to the elevated production of proinflammatory cytokines in patients with CCC.

The BAT1 gene, also known as UAP56, is a member of the DEAD-box family of ATP-dependent RNA helicases [1]. BAT1 is situated in the central region of the major histocompatibility complex (MHC) on the short arm of human chromosome 6 and is ∼40 kb telomeric to the TNFA gene [2]. BAT1 has been shown to down-regulate the inflammatory cytokines tumor necrosis factor (TNF)–α and interleukin-6 in in vitro studies [3]. This observation suggests that BAT1 may play an important role in immunopathological disorders.

Chagas disease, caused by infection with the intracellular protozoan parasite Trypanosoma cruzi, affects ∼16–18 million people in Latin America. Chronic Chagas cardiomyopathy (CCC) develops in nearly 30% of individuals 5–30 years after primary infection, and the remaining unaffected individuals are categorized as having the so-called indeterminate or asymptomatic (ASY) form of the disease. CCC can result in a severe form of dilated cardiomyopathy, and patients with CCC who have severe congestive heart failure have a worse clinical course and shorter survival than do patients who have heart failure of other etiologies without the involvement of a prominent inflammatory component [4, 5].

In patients with CCC, progressive inflammatory events lead to mononuclear-cell infiltration, myocarditis associated with fibrosis, and heart dysfunction [6]. Activated CD8+ T cells are present in the heart tissue of patients with CCC, and TNF-α and interferon (IFN)–γ have been found in biopsy samples and surgical heart-tissue samples from patients with CCC [7]. In 2 studies, higher numbers of IFN-γ–producing cells among peripheral blood mononuclear cells have been found in patients with CCC than in ASY patients [8, 9], whereas 1 study has reported the opposite relationship [10]. High plasma
TNF-α levels have also been reported in patients with CCC, compared with those in ASY patients [11]. The increased circulating levels of TNF-α and elevated TNF-α production by heart-infiltrating mononuclear cells suggests that TNF-α is chronically induced in patients with CCC.

The development of CCC in only approximately one-third of individuals infected with T. cruzi and the tendency for its familial aggregation [12] suggest that genetic factors may be involved in such susceptibility. Better control of T. cruzi infection in the host implies that a fine balance has been achieved between the generation of host immune responses that are sufficient to keep the parasitemia in check and the regulation of the immune response. The mechanism of this balance is poorly understood.

TABLE 1. Baseline characteristics of patients with chronic Chagas cardiomyopathy (CCC) and asymptomatic (ASY) patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ASY (n = 76)</th>
<th>CCC (n = 159)</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Sex</td>
<td>27 (36)</td>
<td>49 (64)</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>53.4 ± 7.9</td>
<td>56.7 ± 9.7</td>
</tr>
<tr>
<td>LVEF ≤40% (n = 72)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEF &gt;40% (n = 80)</td>
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NOTE. Data are no. (%) of patients, unless otherwise indicated. Data on left ventricular ejection fraction (LVEF) were missing for 7 of the patients with CCC.

Patients. Patients with Chagas disease were recruited at the Heart Institute (InCor) of the Hospital das Clinicas, University of São Paulo. Genomic DNA was extracted by the dodecyltrimethyl ammonium bromide/hexadecyltrimethylammonium bromide method. The polymorphisms at positions −22 and −348 in the promoter region of the BAT1 gene were typed by polymerase chain reaction (PCR) restriction fragment–length polymorphism analysis with the restriction enzyme NlaIII (New England Biolabs). The following primer pair flanking both polymorphisms was designed: 5'-CCCTCAGGT-CACCTTCACTACC-3' (BAT1 forward) and 5'-CAACAGCG-ACGAAAGGGAGGA-3' (BAT1 reverse). The BAT1 reverse primer was specifically designed with a T→G substitution to deliberately introduce an NlaIII restriction site when the −22C allele was present in the genomic DNA; the substitution nucleotide is underscored. PCR was performed in a final volume of 25 μL containing 50 ng of genomic DNA, 1.5 mmol/L MgCl₂.

PATIENTS, MATERIALS, AND METHODS

Patients. Patients with Chagas disease were recruited at the Heart Institute (InCor) of the Hospital das Clinicas, University of São Paulo. All of the patients were serologically positive for antibodies against T. cruzi and came from 3 major regions of endemicity. The patients were categorized as being ASY (n = 76) or as having CCC (n = 154) on the basis of clinical, radiological, electrocardiographic (ECG), and echocardiographic criteria. All of the ASY patients had normal ECG findings and a normal left ventricular ejection fraction (LVEF) at the time of echocardiography, as well as normal findings for chest, esophagus, and colon radiography. The patients with CCC presented with abnormal ECG findings and were classified as having moderate (LVEF >40%; n = 80) or severe (LVEF ≤40%; n = 72) CCC. The basic characteristics of the studied patients are shown in table 1.

BAT1 genotyping. Blood samples from the patients with CCC and the ASY patients were collected after they had provided written, informed consent, in accordance with the guidelines of the Internal Review Board of the Hospital das Clinicas, University of São Paulo. Genomic DNA was extracted by the dodecyltrimethyl ammonium bromide/hexadecyltrimethylammonium bromide method. The polymorphisms at positions −22 and −348 in the promoter region of the BAT1 gene were typed by polymerase chain reaction (PCR) restriction fragment–length polymorphism analysis with the restriction enzyme NlaIII (New England Biolabs). The following primer pair flanking both polymorphisms was designed: 5'-CCCTCAGGT-CACCTTCACTACC-3' (BAT1 forward) and 5'-CAACAGCG-ACGAAAGGGAGGA-3' (BAT1 reverse). The BAT1 reverse primer was specifically designed with a T→G substitution to deliberately introduce an NlaIII restriction site when the −22C allele was present in the genomic DNA; the substitution nucleotide is underscored. PCR was performed in a final volume of 25 μL containing 50 ng of genomic DNA, 1.5 mmol/L MgCl₂.

Figure 1. The nt −22 and −348 polymorphisms in the promoter region of the BAT1 gene, as detected by NlaIII restriction endonuclease digestion. Lane 1 shows restriction patterns corresponding to homozygosity for the G allele at nt −22 and for the T allele at nt −348. Lanes 2 and 3 show heterozygosity at nt −348 and homozygosity at nt −22. Lane 4 shows heterozygosity at nt −22. Lane 5 shows homozygosity at nt −22 for the C allele and at nt −348 for the C allele. Lane 6 shows heterozygosity at nt −22 and at nt −348. M, molecular-weight marker.
Table 2. Genotype and allele frequencies for the \textit{BAT1} –22C/G polymorphism in patients with Chagas disease, stratified by those with chronic Chagas cardiomyopathy (CCC) (further stratified by left ventricular ejection fraction [LVEF] status) and those who were asymptomatic (ASY).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>LVEF (\leq 40%) ((n = 72))</th>
<th>LVEF &gt;40% ((n = 75))</th>
<th>All ((n = 154))</th>
<th>ASY ((n = 76))</th>
<th>(\chi^2)</th>
<th>(P_c)</th>
<th>OR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>CC</td>
<td>9 (13)</td>
<td>15 (20)</td>
<td>25 (16)</td>
<td>3 (4)</td>
<td>.004</td>
<td>4.7</td>
<td>(1.4–16)</td>
</tr>
<tr>
<td>CG</td>
<td>37 (51)</td>
<td>37 (49)</td>
<td>74 (48)</td>
<td>35 (46)</td>
<td>.04 (.1)</td>
<td>3.5</td>
<td>(1.0–13)</td>
</tr>
<tr>
<td>GG</td>
<td>26 (36)</td>
<td>23 (31)</td>
<td>55 (36)</td>
<td>38 (50)</td>
<td>.002 (.004)</td>
<td>6.0</td>
<td>(1.7–22)</td>
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Genotype comparison

- CCC vs. ASY: \(\chi^2 = 4.3\), \(P = .019\)
- LVEF \(\leq 40\%\) vs. ASY: \(\chi^2 = 2.9\), \(P = .04 (.08)\)
- LVEF >40\% vs. ASY: \(\chi^2 = 5.9\), \(P = .008 (.02)\)

Allele

<table>
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<tr>
<th>Allele</th>
<th>LVEF (\leq 40%) ((n = 72))</th>
<th>LVEF &gt;40% ((n = 75))</th>
<th>All ((n = 154))</th>
<th>ASY ((n = 76))</th>
<th>(\chi^2)</th>
<th>(P_c)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>55 (38)</td>
<td>67 (45)</td>
<td>124 (40)</td>
<td>41 (27)</td>
<td>7.8</td>
<td>.003</td>
<td>1.8 (1.2–2.8)</td>
</tr>
<tr>
<td>G</td>
<td>89 (62)</td>
<td>83 (65)</td>
<td>184 (60)</td>
<td>111 (73)</td>
<td>10.3</td>
<td>.0007 (.001)</td>
<td>2.2 (1.4–3.5)</td>
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<table>
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<tr>
<th>Allele comparison, C vs. G</th>
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<tr>
<td>CCC vs. ASY</td>
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<tr>
<td>LVEF (\leq 40%) vs. ASY</td>
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<tr>
<td>LVEF &gt;40% vs. ASY</td>
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\(\chi^2\) and \(P_c\) are calculated using the chi-square test and the corrected \(P\) value, respectively.

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**NOTE.** Data are no. (%) of patients, unless otherwise indicated. Data on LVEF were missing for 7 of the patients with CCC. CI, confidence interval; OR, odds ratio; \(P_c\), corrected \(P\) value.

0.25 pmol/L forward primer, 0.25 pmol/L reverse primer, 40 \(\mu\)mol/L dNTP, and 2 \(U\) of \(Taq\) polymerase in buffer containing 100 mmol/L Tris-HCl (pH 8.3) and 500 mmol/L KCl. PCR conditions were an initial denaturation step of 5 min at 95°C; 35 cycles of 20 s at 95°C, 20 s at 62°C, and 30 s at 72°C; and a final step of 7 min at 72°C. The generated PCR product of 429 bp included a nonpolymorphic NlaIII site that served as an internal control for restriction cleavage, providing fragments of 142 bp and 287 bp. When the \(-348T\) allele was present, the 142-bp fragment was cleaved into 81 and 61 bp, whereas, when the \(-22C\) allele was present, the 287-bp fragment was digested into 267 and 20 bp. PCR restriction fragments were size separated by electrophoresis on 10% polyacrylamide gel (figure 1).

**Statistical analysis.** Statistical analysis was done using GraphPad Prism software (version 4.0; GraphPad Software). Genotype and allele frequencies were calculated by direct counting. Hardy-Weinberg equilibrium (HWE) was assessed for the patients with CCC and the ASY patients by comparing the observed number of different genotypes with those expected under HWE for the estimated allele frequency and by comparing the \(\chi^2\) distribution with 2 \(df\). Associations between patient groups and a specific allele as well as between patient groups and genotypes were analyzed by the \(\chi^2\) statistic, along with the relevant odds ratio (OR) and 95% confidence interval (CI). Fisher’s exact test was used when a value in the contingency table was <5. The Bonferroni correction was applied for multiple comparisons.

**RESULTS**

The frequencies of the \(-22C/G\) and \(-348C/T\) polymorphisms of the \textit{BAT1} gene were assessed in 154 patients with CCC and 76 ASY patients. The distribution of genotypes in both groups was under HWE. The genotype and allele frequencies of the \(-22C/G\) polymorphism are given in table 2. A significant difference in frequency between the patients with CCC and the ASY patients was revealed at the genotype level for the \(-22C/G\) polymorphism (\(\chi^2 = 10.0\); \(P = .01\)) —the proportion of \(-22C\) homozygotes among the patients with CCC was 16%, compared with 4% among the ASY patients (\(P = .004\); OR, 4.7 [95% CI, 1.4–16]). When the patients were categorized as those who had the C allele (CC and CG) and those who did not (genotype GG), a statistically significant difference between the patients with CCC and the ASY patients was also found (\(\chi^2 = 4.3\); \(P = .019\)). Thus, the \(-22C\) allele seems to confer susceptibility to CCC. Differences in allele frequencies between
the patients with CCC and the ASY patients were also statistically significant ($P = .003$; OR, 1.8 [95% CI, 1.2–2.8]).

As is shown in table 2, the frequency of −22C genotypes in the patients with CCC was not significantly altered when stratified by LVEF status—no difference was observed between the patients who had an LVEF of ≤40% and those who had an LVEF of >40%. However, there was a significant difference between the patients who had an LVEF of >40% and the ASY patients ($P = .004$; OR, 6.0 [95% CI, 1.7–22]) for −22C homozygosity (20% vs. 4%). The −22C allele was more prevalent among the patients with CCC who had an LVEF of >40% than among the ASY patients, and this difference was statistically significant ($P = .001$; OR, 6.0 [95% CI, 1.4–35]). Only borderline significance was observed at the allelic level for the comparison between the patients with CCC who had an LVEF of ≤40% and the ASY patients ($P = .04$; OR, 1.6 [95% CI, 1.0–2.7]).

Both the genotype and the allele distribution of the BAT1 −348 polymorphism also showed a statistically significant difference between the patients with CCC and the ASY patients, as is shown in table 3. For the CC versus TC plus TT genotype comparison, the OR was 1.9 (95% CI, 1.0–3.5) ($P = .01$). Stratification of the patients by LVEF status revealed a borderline statistically significant difference between the patients with CCC who had an LVEF of ≤40% and the ASY patients for the CC versus TC plus TT comparison ($P = .04$; OR, 2.1 [95% CI, 1.1–4.3]). The OR for the comparison between the patients with CCC who had an LVEF of >40% and the ASY patients showed a similar trend but was not significant (OR, 1.8 [95% CI, 0.9–3.6]). The OR for the C versus T allele comparison for −348C between the patients with CCC and the ASY patients was 1.7 (95% CI, 1.0–2.8) ($P = .02$). A similar OR trend was observed when the patients with CCC were stratified by LVEF status but failed to reach statistical significance after application of the Bonferroni correction.

## DISCUSSION

To the best of our knowledge, this is the first study undertaken to examine the potential influence that the BAT1 gene promoter polymorphisms −22C/G and −348C/T have on the clinical course of Chagas disease. The major finding is an association between the −22C/C and −348C/T polymorphisms of BAT1 and the risk of developing CCC. Patients homozygous for the −22C variant showed a 5-fold greater risk of developing CCC, compared with patients with either 1 or 0 copies of the risk allele. Similarly, patients homozygous for the −348C allele showed a 2-fold greater risk of developing CCC.

In a previous study, the BAT1 promoter region (−22C and −348C) found in the disease-associated 8.1 ancestral haplotype (AH) (HLA-A1, -B8, and -DR3) consistently showed lower reporter-gene transcriptional activity, compared with the disease-resistant 7.1 AH (HLA-A3, -B7, and -DR15), which carries the −22G and −348T variants [14]. Nuclear extracts from T cells, monocytes, and epithelial cells bind to the region spanning the −22G and −348T positions, enabling the identification of...
transcription factors that bind differentially to the 2 alleles [13]. In a transfection reporter assay, the fragment containing the −22G and −348T sequences was expressed more efficiently than the fragment containing the −22C and −348C sequences [13]. In light of these data, it can be assumed that individual carriage of either the −22G or the −348T allele is likely to be associated with an attenuated inflammatory response, compared with that in bearers of the −22C or −348C variant. Of note, the −348T variant is in linkage disequilibrium with the −22G variant and is found only on chromosomes bearing the −22G variant. Furthermore, the −22C and −348C variants are both found on the 8.1 AH [13], which has been associated with high TNF-α production [15, 16].

We previously reported that there was no association between the risk of developing CCC and 2 HLA class II genes (HLA-DR and HLA-DQ) in the Brazilian population [17]. There have been studies that have screened for the TNF-α −238 and −308 promoter polymorphisms in patients with Chagas disease. One study reported no association between the risk of developing CCC and these TNF-α promoter polymorphisms in the Peruvian population [18], whereas another cited an association with the risk of developing CCC in the Mexican population [19]. Progression to chronic Chagas disease is very likely a multifactorial process that depends on the combination of host genetic factors and environmental factors, with each gene contributing slightly to the pathogenesis of the disease. Our present results suggest that BAT1 polymorphisms may be one of these host genetic factors. These polymorphisms either are functionally associated with the development of CCC and, thus, reflect a true association or are in linkage disequilibrium with a critical gene.

Inflammatory-cell infiltrates have been described in autopsied hearts of patients with the ASY form [20], but recent biopsy studies have shown that patients with CCC who experience heart failure are more likely to have severe myocarditis, fibrosis, and myocardial hypertrophy, suggesting a key role for myocardial inflammation [21–23]. Hence, progression to overt cardiomyopathy may result from the combined effect of high expression of proinflammatory cytokines [7, 8, 24] triggered by parasite persistence and inadequate counterregulation of these cytokines. Because BAT1 may be a negative regulator of proinflammatory cytokines, we propose that the increased risk for developing CCC among −22C and −348C homozygotes reflects an uncontrolled production of inflammatory cytokines that leads to cardiac-tissue damage in these subjects. Indeed, BAT1 appears to be a fast-acting down-regulator of acute-phase cytokines, with a prominent effect on TNF-α [3].

The overrepresentation of the −22C variant among the patients with CCC who had an LVEF of >40% can be explained by its coding for low transcriptional activity. On the other hand, the lower prevalence of −22C homozygotes among the patients with CCC who had an LVEF of ≤40% (even though it was still higher than that among the ASY patients) might reflect the severity of the disease and selection through the deaths of these patients via this or other intervening susceptibility factors, such as TNF-α production. Indeed, it was recently reported that, in the Brazilian population, Chagas heart disease was associated with the highest risk of death, compared with the risk in patients with heart failure of different etiologies [25]. It can be assumed that we missed these patients with severe disease because of social deprivation [26], and this might have caused a paucity of patients in that particular cohort subset.

In summary, our results indicate that the −22C and −348C promoter polymorphisms in the BAT1 gene are risk factors for the development of CCC in the Brazilian population. This finding sheds new light on the pathogenic mechanism of the disease and raises a potentially interesting issue that is worthy of further study in other populations.

References


