RESEARCH ARTICLE

**IL18 Gene Variants Influence the Susceptibility to Chagas Disease**

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**Abstract**

Chagas disease is a parasitic disorder caused by the infection with the flagellated protozoan *Trypanosoma cruzi*. According to the World Health Organization, more than six million people are currently infected in endemic regions. Genetic factors have been proposed to influence predisposition to infection and development of severe clinical phenotypes like chronic Chagas cardiomyopathy (CCC). Interleukin 18 (*IL18*) encodes a proinflammatory cytokine that has been proposed to be involved in controlling *T. cruzi* infection. In this study, we analyzed the possible role of six *IL18* gene variants (rs5744258, rs360722, rs2043055, rs187238, rs1946518 and rs360719), which cover most of the variation within the locus, in the susceptibility to infection by *T. cruzi* and/or CCC. In total, 1,171 individuals from a Colombian region endemic for Chagas disease, classified as seronegative (n = 595), seropositive asymptomatic (n = 175) and CCC (n = 401), were genotyped using TaqMan probes. Significant associations with *T. cruzi* infection were observed when comparing seronegative and seropositive individuals for rs187238 (P = 2.18E-03, OR = 0.77), rs360719 (P = 1.49E-03, OR = 0.76), rs2043055 (P = 2.52E-03, OR = 1.29), and rs1946518 (P = 0.0162, OR = 1.22). However, dependence analyses suggested that the association was mainly driven by the polymorphism rs360719. This variant is located within the promoter region of the *IL18* gene, and it has been described that it creates a binding site for the transcription factor OCT-1 affecting IL-18 expression levels. In addition, no evidence of association was observed between any of the analyzed *IL18* gene polymorphisms and the development of CCC. In summary, our data suggest that genetic variation within the promoter region of *IL18* is directly involved in the susceptibility to infection by *T. cruzi*, which provides novel insight into disease pathophysiology and adds new perspectives to achieve a more effective disease control.
Author Summary

Chagas disease is a parasitic disorder caused by the infection with the protozoan Trypanosoma cruzi. In Latin America, this disease represents a major public health concern, as almost 6 million people are currently infected. During the last years, great efforts have been made in health policy to control the disease; however, there is still a long way ahead to achieve this challenging goal. Most affected people remains asymptomatic after infection for the rest of their lives, but around one third of infected people may develop cardiomyopathy, a condition that reduces dramatically the quality of life and life expectancy in Chagas patients. The causes of the marked differential disease outcomes are currently unknown, but it is believed that a genetic predisposition could play a relevant role in the host. We investigated in an endemic region of Colombia whether the IL18 gene, which is involved in the immune response to intracellular pathogens like T. cruzi, is related to a higher susceptibility to infection or disease severity. Our results suggest that IL18 is a relevant gene in Chagas disease, and could represent a valuable insight that may help to better understand the disease pathogenesis and the development of more efficient therapeutic strategies.

Introduction

Host genetic factors have been suggested to play an important role in the susceptibility to human infectious diseases [1]. An example of such conditions is Chagas disease, which is caused by infection of the protozoan Trypanosoma cruzi. Recent estimations indicate that more than 70 million people live in endemic areas for this parasite, with around 6 million people being currently infected and a reported incidence of the disease of almost 30,000 cases [2,3]. Two phases, acute and chronic, are clearly defined in Chagas disease. The early stages are characterized by acute symptoms like fever, headache or swollen lymph nodes. After 8–12 weeks from the bite, infected individuals enter the chronic phase of the disease, in which most of them will remain asymptomatic for the rest of their lives. However, around 30% of patients will develop further symptoms, including chronic cardiomyopathy and/or digestive complications [4]. During the last decade, several studies have investigated the possible role of gene polymorphisms in the predisposition to T. cruzi infection and/or chronic Chagas cardiomyopathy in patients from endemic countries, reporting promising results [5–17].

Interleukin 18 (IL18) is one of the genes that have been proposed to influence the development of Chagas disease. It encodes a proinflammatory cytokine that was originally described as an interferon-gamma (IFN-γ) inducing factor. Because of this, IL-18 was classified among the Th1-inducing family of cytokines, along with IL-2, IL-12 and IL-15 [18]. Due to its crucial role in the induction of IFN-γ production by T cells and NK cells, thus promoting the Th1 response, IL-18 is considered a relevant molecule for controlling intracellular pathogens [19,20].

In Chagas disease, IFN-γ is essential for parasite control during the early stages of the infection. It has been described that knockout mice for IFNG are highly susceptible to infection due to defective macrophage activation and nitric oxide production [21]. Interestingly, mice inoculated with T. cruzi displayed elevated IL-18 levels 6 days after infection followed by an increase of IL-12 and IFNγ [22]. Indeed, IL-18 can mediate IFN-γ induction in T cells in an IL-12 independent manner [23].

Consistent with the above, previous studies have suggested a genetic influence of both IFNG and IL18 gene variants in the susceptibility to infection by T. cruzi and Chagas cardiomyopathy,
respectively [9,15], adding additional evidences to the high relevance that this pathway may have in Chagas disease development.

Taking into consideration all this knowledge, we decided to perform a comprehensive analysis of the IL18 variation, in a well-powered cohort from an endemic region of T. cruzi, in order to dissect the possible genetic association of the region with predisposition to infection by this parasite and/or the development of cardiomyopathy in Chagas patients.

Materials and Methods

Study subjects

A total of 1,171 Colombian individuals from an endemic region for Chagas disease (Guanetina and Comunera provinces, at the department of Santander localized between 5°26' and 8° 08’ north and 72°26' and 74°32’ west) were included in this study (S1 Fig). The population in this region of Colombia is a homogeneous mixture, with no specific concentration of any ethnicity. All participants underwent a serological diagnosis for T. cruzi infection by means of the enzyme-linked immunosorbent assay (ELISA) and a commercial indirect hemagglutination test. According to the results of these tests, 576 individuals were classified as seropositive for T. cruzi antigens and 595 were classified as seronegative, with this latter group being used as controls. Subsequently, and based on the results of the clinical evaluation, an electrocardiogram and echocardiogram were recorded to detect any conduction alteration and/or structural cardiomyopathy. As a result, 175 seropositive individuals were classified as asymptomatic and 401 individuals were classified as having chronic Chagas cardiomyopathy. From this last group, Chagas patients were further subclassified accordingly to the severity of cardiomyopathy as follows: CII (n = 166, radiology indicative of light heart hypertrophy or minor ECG alterations), CIII (n = 200, moderate heart hypertrophy and considerable ECG alterations, mainly conduction abnormalities) and CIV (n = 35, severe cardiomegaly and marked ECG alterations, predominantly frequent and/or complex forms of ventricular arrhythmia). The mean age of participants was 45.86 years for seronegative individuals, 58.00 for asymptomatic individuals and 63.14 for chronic Chagas cardiomyopathy patients. The sex distribution for the entire group was 55% female and 45% male. None of the patients included in this study received any treatment (i.e. Benznidazole) for the infection.

Ethics statement

The Ethics Committees from the ‘Universidad Industrial de Santander and Fundación Cardiovascular de Colombia’ approved this study (entitled “Identificación de factores de riesgo genético para Cardiopatía Chagásica crónica” [Identification of genetic risk factors for chronic Chagas cardiomyopathy] and approved on June 27th 2005 in the Act No. 15 of 2005) in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation.

SNP selection and genotyping

In order to comprehensively analyze the possible role of IL18 on the genetic susceptibility to Chagas disease, a total of six single-nucleotide polymorphisms (SNP) within the locus were selected for genotyping following a combined candidate gene/tagging strategy. These include: 1) two promoter variants (rs187238 and rs1946518) that have been reported to affect gene expression [24–27]; 2) one intronic variant (rs2043055) previously implicated in Chagas disease outcome in a Brazilian population [15]; 3) an additional promoter variant (rs360719) that has been described to interact with the transcription factor OCT-1 [28]; and 4) two intronic tag
SNPs (rs5744258 and rs360722) covering the remaining variability of the IL18 gene. These latter variants were selected with the software Haploview V4.2 [29] on the basis of both pairwise tagging ($r^2 > 0.80$) and minor allele frequencies $>0.1$, using Colombian-Medellín (CLM) genotype data from the 1000 genomes phase III project (http://www.1000genomes.org) [30], encompassing the coding and promoter regions of IL18 and considering the four previously selected candidate variants.

Genomic DNA was isolated following standard procedures and the genotyping was performed using TaqMan assays (Applied Biosystems, Foster City, California, USA) on a LightCycler 480 real-time PCR system (Roche Diagnostics, Basel, Switzerland).

Statistical analysis

All statistical analyses were performed with the statistical software package Plink V1.07 (http://pngu.mgh.harvard.edu/purcell/plink) [31]. Deviance from Hardy-Weinberg equilibrium was determined at the 1% significance level in all groups of individuals. To test for possible allelic and genotypic associations, we analyzed the allelic, genotypic and haplotypic frequencies by comparing seronegative vs. seropositive individuals and asymptomatic vs. chronic Chagas cardiomyopathy individuals using the $\chi^2$ test and logistic regression analyses, when necessary. The Benjamini & Hochberg step-up false discovery rate (FDR) correction was used in all analyses to control for multiple testing. Permutation tests (10,000 permutations) were also performed in the haplotype analysis to estimate empirical P-values as implemented in Plink. Odds ratios (OR) and 95% confidence intervals (CI) were calculated according to the Woolf’s method. P-values lower than 0.05 were considered as statistically significant. Pairwise linkage disequilibrium (LD) ($D’$ and $r^2$) and haplotypes were estimated using an expectation–maximization algorithm implemented in Haplovew. In addition, to determine whether the haplotype model better explained the observed effects than the model considering the individual SNPs, we compared the goodness of fit of both models by a likelihood ratio test as described elsewhere [32].

The statistical power of our study (Table A in S1 Text) was estimated with the Power Calculator for Genetic Studies 2006 (CaTS) software (http://www.sph.umich.edu/csg/abecasis/CaTS/) [33].

Results

The six IL18 SNPs were in Hardy-Weinberg equilibrium in all the analyzed subgroups ($P > 0.01$), suggesting that a possible inbreed in Guanentina and Comunera provinces is not likely. The genotyping success rate was over 95% and the allele frequencies in all cases were similar to those described for the Colombian population (CLM) of the 1000 genomes phase III project (http://www.1000genomes.org) [30]. A relatively high LD was observed throughout the gene in the analyzed population (Fig 1). Particularly, rs187238 and rs360719 showed an $r^2$ value = 0.98, indicating that these two variants are almost completely linked and, consequently, they may be considered as the same marker for this study.

In order to evaluate the possible association between IL18 gene variants and susceptibility to T. cruzi infection, the allelic and genotypic frequencies of seronegative and seropositive individuals were compared (Table 1). The allelic frequencies of four out of the six IL18 genetic variants were significantly different between these two groups of individuals. In this regard, rs187238°C and rs360719°C were significantly increased in seronegative individuals compared to the seropositive subset ($P = 2.18E-03$, $P_{FDR} = 5.04E-03$, OR = 0.77, CI = 0.65–0.91; and $P = 1.49E-03$, $P_{FDR} = 5.04E-03$, OR = 0.76, CI = 0.64–0.90; respectively), thus suggesting that these variants are associated to protection against infection by T. cruzi. On the contrary, the
frequencies of rs2043055 C and rs1946518 C were reduced in the seronegative sample set in comparison with the seropositive one (P = 2.52E-03, PFDR = 5.04E-03, OR = 1.29, CI = 1.10–1.53; and P = 0.0162, PFDR = 0.0243, OR = 1.22, CI = 1.04–1.44; respectively), indicating that they are associated with a higher infection risk. No statistical significance was observed when the allelic and genotypic frequencies of both rs5744258 and rs360722 were compared between seropositive and seronegative individuals.

The marked difference of the average age between the different subgroups of patients (i.e., seronegative and seropositive individuals) could represent a limitation in this study, as Chagas disease is a parasitic disorder in which patients could develop symptoms many years after the infection [3,4]. To control for this possible confounding factor, we decided to perform a logistic regression analysis accordingly with the serological status using age as covariate. The statistical significance was maintained in this analysis thus supporting the consistency of our results (Table B in S1 Text).

Due to the high LD among the four associated SNPs, which are located either within or nearby the promoter region, dependency of the associations could be masking a possible unique causal variant. To check this, conditional logistic regression analyses were conducted.
by conditioning each associated SNP to the remaining variants (except for rs187238 that was almost completely dependent to rs360719). The results of these analyses pointed to rs360719 as the most likely causative variant of the \textit{IL18} association with Chagas disease, as the statistical significance of both rs2043055 and rs1946518 were clearly lost after conditioning to it, and a trend was maintained when conditioning rs360719 on these two latter variants (Table 2).

Subsequently, in order to investigate the possible association between \textit{IL18} and chronic Chagas cardiomyopathy, we compared the allelic and genotypic frequencies of the \textit{IL18} SNPs between seropositive asymptomatic individuals and chronic Chagas cardiomyopathy individuals (Table 3). No statistically significant differences were observed between asymptomatic individuals and chronic Chagasic cardiomyopathy patients for any of the analyzed polymorphisms. In addition, to further evaluate the possible association between \textit{IL18} and progression of cardiomyopathy, we compared \textit{IL18} allelic and genotypic frequencies by grouping asymptomatic + CII individuals and CIII + CIV individuals; however, similar to that observed in the previous analysis, no statistically significant differences were yielded (Table C in S1 Text).

Finally, we also investigated a possible haplotype effect between the associated SNPs and susceptibility to \textit{T. cruzi} infection (Table 4). Five possible haplotypes were observed (rs2043055|rs187238|rs1946518|rs360719: TCAC, CGCT, TGCT, TGAT and CGAT), with the haplotypes TCAC and CGCT showing the higher frequencies (37.30% and 37.40%, respectively). In relation to the seronegative vs seropositive analysis, the frequency of TCAC was increased in the former individuals, being this difference statistically significant ($P = 1.50E-03$, $P_{FDR} = 7.50E-03$, $OR = 0.76$, CI = 0.65–0.90). On the other hand, the frequency of CGCT was

| SNP     | P-value | P-value add to rs360719 | P-value add to rs2043055 | P-value add to rs1946518 |
|---------|---------|-------------------------|--------------------------|--------------------------|
| rs360719 | 1.49E-03 | NA                      | 0.0996                   | 0.0548                   |
| rs2043055 | 2.52E-03 | 0.1839                  | NA                       | 0.1035                   |
| rs1946518 | 0.0162   | 0.8918                  | 0.6340                   | NA                       |

\textsuperscript{‡} Same signal as rs187238

doi:10.1371/journal.pntd.0004583.t002

| SNP        | Genotype. N (%) | MAF % | P     | OR [95% CI] |
|------------|-----------------|-------|-------|-------------|
| rs5744258  | C|G Asymptomatic (175) | 0 (0.00) | 37 (21.14) | 138 (78.86) | 10.57 |
|            | CCC (395)       | 2 (0.51) | 91 (23.04) | 302 (76.46) | 12.03 |
| rs360722   | T|C Asymptomatic (174) | 4 (2.30) | 29 (16.67) | 141 (81.03) | 10.63 |
|            | CCC (398)       | 7 (1.75) | 90 (22.56) | 301 (75.69) | 13.07 |
| rs2043055  | C|T Asymptomatic (173) | 36 (20.81) | 78 (45.09) | 59 (34.01)  | 43.35 |
|            | CCC (395)       | 62 (15.70) | 203 (51.39) | 130 (32.91) | 41.39 |
| rs187238   | C|G Asymptomatic (173) | 25 (14.45) | 74 (42.77) | 74 (42.77)  | 35.84 |
|            | CCC (399)       | 42 (10.53) | 185 (46.37) | 172 (43.11) | 33.71 |
| rs1946518  | A|C Asymptomatic (175) | 45 (25.71) | 80 (45.71) | 50 (28.57)  | 48.57 |
|            | CCC (397)       | 87 (21.91) | 203 (51.13) | 107 (26.95) | 47.48 |
| rs360719   | C|T Asymptomatic (174) | 26 (14.94) | 75 (43.10) | 73 (41.95)  | 36.49 |
|            | CCC (398)       | 41 (10.30) | 183 (45.98) | 174 (43.72) | 33.29 |

doi:10.1371/journal.pntd.0004583.t003
significantly lower in seronegative individuals compared with seropositive individuals ($P = 0.0116$, $PFDR = 0.0290$, $OR = 1.25$, $CI = 1.05–1.47$), whereas the frequencies of TGCT, TGAT, and CGAT did not differ significantly between seropositive and seronegative individuals. Similar results were observed when the haplotype analysis was performed using permutation test with 10,000 permutations instead of Chi-square (Table 4). However, the haplotype model did not better explain the $IL18$ association to risk of infection than the model considering the SNPs independently (likelihood $P$-value = 0.1454), indicating no additive effects (that is, the associated haplotypes were a consequence of the independent associations of the considered variants).

In relation to the haplotype analysis according to the presence/absence of chronic Chagas cardiomyopathy and/or to the progression of cardiomyopathy, no statistically significant differences among different subgroups of individuals were observed (Tables D and E in S1 Text).

**Discussion**

This study evidenced that four genetic variants, namely rs2043055, rs187238, rs1946518 and rs360719, are statistically associated to differential risk of infection by $T. cruzi$ in a Colombian population. However, our data suggested that the association is mainly driven by a single SNP, likely rs360719. Evidences supporting this fact include: 1) this $IL18$ variant showed the most significant $P$-value and the higher effect size; 2) the statistical significance of both rs2043055 and rs1946518 was lost after conditioning on rs360719, whereas a trend towards significance was clearly observed for rs360719 after conditioning on rs2043055 or rs1946518; 3) no improvement in the goodness of fit for the model considering the association with rs360719 was observed for any of the haplotypic models; and 4) this $IL18$ variant has a demonstrated functional implication in the gene expression [28].

On the other hand, $IL18$ does not seem to be involved in later parasitic burden in the tissues of chronic infected patients. It should be noted that the analysis between symptomatic and asymptomatic patients was performed with lower statistical power than that between seronegative and seropositive patients (S1 Table). Hence, a possible type II error may not be ruled out. Another possibility could be that additional genetic/environmental factors other than this gene may have a higher relevance for the disease progression [5–7,11–14].

In addition, a lack of association with infection by $T. cruzi$ was observed for rs5744258 and rs360722. These two polymorphisms had the lower minor allele frequency and, therefore, their analysis could be limited in terms of statistical power. However, the power considering our study cohort was not reduced (92% to detect associations with $OR = 1.5$ at the 5% significance level).

Table 4. $IL18$ haplotype analysis of seropositive and seronegative individuals.

| Haplotype | Seropositive | Seronegative | $P$ | $P_{FDR}$ | $P_{PERM}$ | OR [95% CI] |
|-----------|--------------|--------------|-----|-----------|------------|-------------|
| TCAC      | 391 (34.10)  | 479 (40.50)  | 1.50E-03 | 7.50E-03  | 4.90E-03   | 0.76 [0.65–0.90] |
| CGCT      | 458 (40.00)  | 413 (35.00)  | 0.0116  | 0.0290    | 0.0428     | 1.25 [1.05–1.47] |
| TGCT      | 138 (12.10)  | 144 (12.20)  | 0.9222  | 0.9222    | 1.00       | 0.99 [0.77–1.27] |
| TGAT      | 137 (12.00)  | 134 (11.30)  | 0.6148  | 0.7685    | 0.9758     | 1.00 [0.89–1.12] |
| CGAT      | 18 (1.60)    | 12 (1.00)    | 0.2262  | 0.3770    | 0.6380     | 1.52 [0.74–3.13] |

‡Order of SNPs: rs2043055|rs187238|rs1946518|rs360719

*P value after Benjamini & Hochberg step-up false discovery rate correction.

**Permutation test $P$-value for 10,000 permutations.

doi:10.1371/journal.pntd.0004583.l004
and the allele frequencies of the tested groups were very similar (rs5744258: 11.57% vs 11.58%, OR = 1.00; rs360722: 11.59% vs 12.33%, OR = 1.07). Analysis of larger cohorts would be required to definitively discard these IL18 variants as susceptibility markers for Chagas disease.

A possible limitation in the inclusion methodology of our study could be that the seronegative group comprised individuals that underwent a seroconversion. However, in our opinion, it is more likely that seronegative individuals with putative spontaneous cure avoided antibody production due to a quick innate immune response by killer cells and macrophages instead. No consistent seroconversion rates have been reported in Chagas patients so far, and seroconverted individuals were reported only after treatment when there is not persistence in the infection [34–36]. None of the seronegative individuals included in our study were either reported to have Chagas disease or to have a previous therapy.

In addition, it should be noted that there is a considerable high prevalence of cardiac patients in our study cohort, which could suggest that the seropositive population is biased to the patients with Chagas cardiomyopathy. However, we would like to state that the participants were recruited after a medical visit to the endemic area. In this regard, individuals coming to the citation underwent serological analyses, and those showing seropositivity were subsequently subjected to electrocardiograms and further medical analyses in which they were classified as asymptomatic seropositive or CCC patients. In any case, this sample set has been used in previously published studies by our group [13] and we are confident about its homogeneity.

IL-18 is a cytokine which induces IFN-γ production activating several immune cells in response to intracellular pathogens, including T. cruzi [20]. IL-18 was shown to play an important role in early immunity to Chagas disease [22,23]. Moreover, the susceptibility to T. cruzi depends on the capability of releasing IFN-γ during early stages of infection and this is directly related to release of IL-18 during this phase [37]. Regarding this, it has been reported that rs360719 may be located within a repressor site of the gene, and individuals carrying the C allele showed a higher IL18 expression due to the creation of a binding site for the transcription factor OCT-1 [28]. This is consistent with the protective role that we observed for this allele in Chagas disease development, and support the hypothesis that major IL-18 levels could increase parasite clearance in early stages of infection. Besides, our results are also in concordance with a previous study performed by our group reporting an association between IFNG and susceptibility to infection by T. cruzi [9]. Altogether, these findings clearly point to IL-18 along with IFN-γ as crucial players in the immune response against infection by this parasite. In any case, additional functional analyses are needed to confirm this assumption, and to have an accurate estimation of the putative IL-18 and IFN-γ levels that may discriminate the different subgroups of Chagas patients from each other and from the healthy population.

On the other hand, a previous study showed that IL18 rs2043055 may modulate Chagas disease severity in a Brazilian population [15]. In our study we were not able to find an association of any of the analyzed IL18 SNPs (including this one) with the severity of Chagas disease. We speculate that the discrepancy could be due to a different genetic background between the analyzed cohorts from Brazil and Colombia. Despite being both populations a mixture from Amerindian, west-European and African populations, the proportion of these ancestries could differ between them, which would affect the LD and haplotypic block architecture across the genome [38–40]. It would be interesting, therefore, to examine whether the association described for rs2043055 is dependent upon rs360719 in the Brazilian population, as our data suggest based on the LD structure observed in our Colombian cohort. In any case, both studies open a new window to understand differences in Chagas disease outcome and susceptibility.

Another explanation for the observed differences between both studies could be the existence of different T. cruzi strains in the studied regions from Colombia and Brazil, as the two strains present in such areas (I and II, respectively) have been described to be implicated in CCC.
development and severity [41]. The analysis of the specific strains affecting both our population and the Brazilian one was out of the scope of this study, but it could represent an interesting future complementary analysis to this reported here.

The influence of IL18 gene variants on the susceptibility to infection or severity of other protozoan infectious diseases has been also evaluated. In this context, a weak association between the IL18 SNP rs1946519, and a higher risk to develop Leishmaniasis was described in Iranians [42]. Nevertheless, the authors did not find evidence of association between Leishmania infection and rs187238, which was associated with T. cruzi infection in our study. As stated before, the discrepancy could be due to population-specific genetic architectures within the gene, but also to the fact that our study had a considerably higher statistical power.

Additionally, the possible role of the IL18 gene variants rs187238 and rs1946518 in severe malaria anemia and mortality were also investigated in a Kenyan children population [43]. The authors of that study observed that homozygosity for the rs1946518’A allele conferred protection against severe malaria, and that the allelic combination of rs187238’G and rs19463518’C had a higher frequency in the severe malaria group compared to the non-severe group [43]. In our study, the frequency of the AA genotype for rs1946518 was increased in asymptomatic individuals compared to chronic Chagas cardiomyopathy patients, but this difference was not statistically significant. Similarly, the frequency of the rs187238’G|rs19463518’C haplotype was increased in chronic cardiomyopathy individuals compared with asymptomatic patients, although the difference did not reach statistical significance either. Additional studies encompassing larger cohorts of seropositive patients with different degrees of disease severity may shed light into these putative associations.

IL18 gene variants have also been evaluated in other infectious conditions such as hepatitis B or C viruses. Cumulating data indicate that IL-18 may influence the clearing of the viral load [44–46], as well as the severity of the infection in some cases of hepatic carcinomas or cirrhosis [26,47,48]. In this context, it has been proposed that differential expression levels of IL18 could be directly involved in the predisposition to infection by the above mentioned viruses and in the severity of hepatitis [26,44–48], consistent with what we observed in Chagasic patients.

In conclusion, our results suggest that IL18 variation plays an important role in the susceptibility to infection by T. cruzi, probably by influencing IL-18 production during the immune response in the early stages of the infection. The promoter polymorphism rs360719 is likely the causal variant of this association, at least in the Colombian population. In any case, further studies on this gene on different ancestries and larger samples sizes, as well functional analyses, would be desirable to validate our findings.

Supporting Information

S1 Fig. Location of the Colombian endemic regions analyzed in this study (Guanentina and Comunera).

(TIF)

S1 Text. Supporting information tables. Table A. Statistical power calculation considering different effect sizes; Table B. Logistic regression analysis of IL18 polymorphisms in seronegative and seropositive individuals including age as covariate; Table C. Genotype and allele distribution for IL18 polymorphisms in early chronic Chagas cardiomyopathy (Asymptomatic + CII) and advanced chronic Chagas cardiomyopathy (CIII+CIV) individuals; Table D. IL18 haplotype analysis of asymptomatic and chronic Chagas cardiomyopathy individuals; Table E. IL18 haplotype analysis of early chronic Chagas cardiomyopathy (Asymptomatic + CII) and advanced chronic Chagas cardiomyopathy (CIII+CIV) individuals.

(DOCX)
Acknowledgments

This work is part of the doctoral thesis "Estudio de las bases genéticas de la enfermedad de Chagas" from the Biomedicine PhD program at the Universidad de Granada (Spain).

Author Contributions

Conceived and designed the experiments: DALR FDC JM. Performed the experiments: DALR. Analyzed the data: DALR FDC. Contributed reagents/materials/analysis tools: CIG LEE JM. Wrote the paper: DALR FDC CIG JM.

References

1. Chapman SJ, Hill AV (2012) Human genetic susceptibility to infectious disease. Nat Rev Genet 13: 175–188. doi: 10.1038/nrg3114 PMID: 22310894
2. (2015) Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec 90: 33–43. PMID: 25671846
3. Bern C (2015) Chagas' Disease. N Engl J Med 373: 456–466. doi: 10.1056/NEJMra1410150 PMID: 26222561
4. Rassi A Jr., Rassi A, Marin-Neto JA (2010) Chagas disease. Lancet 375: 1388–1402. doi: 10.1016/S0140-6736(10)60061-X PMID: 20399979
5. Calzada JE, Nieto A, Beraun Y, Martin J (2001) Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. Tissue Antigens 58: 154–158. PMID: 11703822
6. Florez O, Zafra G, Morillo C, Martin J, Gonzalez CI (2006) Interleukin-1 gene cluster polymorphism in chagas disease in a Colombian case-control study. Hum Immunol 67: 741–748. PMID: 17002905
7. Zafra G, Morillo C, Martin J, Gonzalez A, Gonzalez CI (2007) Polymorphism in the 3' UTR of the IL12B gene is associated with Chagas' disease cardiomyopathy. Microbes Infect 9: 1049–1052. PMID: 17644387
8. Calzada JE, Beraun Y, Gonzalez CI, Martin J (2009) Transforming growth factor beta 1 (TGFb1) gene polymorphisms and Chagas disease susceptibility in Peruvian and Colombian patients. Cytokine 45: 149–153. doi: 10.1016/j.cyto.2008.11.013 PMID: 19136278
9. Torres OA, Calzada JE, Beraun Y, Morillo CA, Gonzalez A, et al. (2010) Role of the IFNG +874T/A polymorphism in Chagas disease in a Colombian population. Infect Genet Evol 10: 682–685. doi: 10.1016/j.meegid.2010.03.009 PMID: 20359550
10. Florez O, Martin J, Gonzalez CI (2012) Genetic variants in the chemokines and chemokine receptors in Chagas disease. Hum Immunol 73: 852–858. doi: 10.1016/j.humimm.2012.04.005 PMID: 22537745
11. Criado L, Florez O, Martin J, Gonzalez CI (2012) Genetic polymorphisms in TNFA/TNFR2 genes and Chagas disease in a Colombian endemic population. Cytokine 57: 398–401. doi: 10.1016/j.cyto.2011.12.007 PMID: 22221522
12. Machuca MA, Suarez EU, Echeverria LE, Martin J, Gonzalez CI (2014) SNP/haplotype associations of CCR2 and CCR5 genes with severity of chagasic cardiomyopathy. Hum Immunol 75: 1210–1215. doi: 10.1016/j.humimm.2014.09.023 PMID: 25312802
13. Leon Rodriguez DA, Echeverria LE, Gonzalez CI, Martin J (2015) Investigation of the role of IL17A gene variants in Chagas disease. Genes Immun 16: 536–540. doi: 10.1038/gene.2015.42 PMID: 26468780
14. Cunha-Neto E, Chevillard C (2014) Chagas disease cardiomyopathy: immunopathology and genetics. Mediators Inflamm 2014: 683230. doi: 10.1155/2014/683230 PMID: 25210230
15. Nogueira LG, Frade AF, Laini BM, Laugier L, Pissetti CW, et al. (2015) Functional IL18 polymorphism and susceptibility to Chronic Chagas Disease. Cytokine 73: 79–83. doi: 10.1016/j.cyto.2015.01.037 PMID: 25743241
16. Vasconcelos RH, Montenegro SM, Azevedo EA, Gomes YM, Morais CN (2012) Genetic susceptibility to chronic Chagas disease: an overview of single nucleotide polymorphisms of cytokine genes. Cytokine 59: 203–208. doi: 10.1016/j.cyto.2012.04.035 PMID: 22935647
17. Ayo CM, Dalalio MM, Visentainer JE, Reis PG, Sippert EA, et al. (2013) Genetic susceptibility to Chagas disease: an overview about the infection and about the association between disease and the immune response genes. Biomed Res Int 2013: 284729. doi: 10.1155/2013/284729 PMID: 24089594
18. Dinarello CA (1999) IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 103: 11–24. PMID: 9999178
19. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 12: 53–72. PMID: 11312119

20. Sugawara I (2000) Interleukin-18 (IL-18) and infectious diseases, with special emphasis on diseases induced by intracellular pathogens. Microbes Infect 2: 1257–1263. PMID: 11008115

21. Holscher C, Kohler G, Muller U, Mossmann H, Schaub GA, et al. (1998) Defective nitric oxide effector functions lead to extreme susceptibility of Trypanosoma cruzi-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. Infect Immun 66: 1208–1215. PMID: 9488415

22. Meyer Zum Buschenfelde C, Cramer S, Trumpfheller C, Fleischer B, Frosch S (1997) Trypanosoma cruzi induces strong IL-12 and IL-18 gene expression in vivo: correlation with interferon-gamma (IFN-gamma) production. Clin Exp Immunol 110: 378–385. PMID: 9409639

23. Muller U, Kohler G, Mossmann H, Schaub GA, Alber G, et al. (2001) IL-12-independent IFN-gamma production by T cells in experimental Chagas’ disease is mediated by IL-18. J Immunol 167: 3346–3353. PMID: 11544324

24. Thompson SR, Humphries SE (2007) Interleukin-18 genetics and inflammatory disease susceptibility. Genes Immun 8: 91–99. PMID: 17215860

25. Giedraitis V, He B, Huang WX, Hillert J (2001) Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. J Neuroimmunol 112: 146–152. PMID: 11108943

26. Bouzgarrou N, Hassen E, Schvoerer E, Stoll-Keller F, Bahri O, et al. (2008) Association of interleukin-18 polymorphisms and plasma level with the outcome of chronic HCV infection. J Med Virol 80: 607–614. doi: 10.1002/jmv.21079 PMID: 1842334

27. Skol AD, Scott LJ, Abecasis GR (2006) Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38: 209–213. PMID: 16415888

28. Escriba JM, Ponce E, Romero Ade D, Vinas PA, Marchiol A, et al. (2009) Treatment and seroconversion in a cohort of children suffering from recent chronic Chagas infection in Yoro, Honduras. Mem Inst Oswaldo Cruz 104: 986–991. PMID: 20027465

29. Alvarado MG, Vigiliano C, Lococo B, Petti M, Bertocchi G, et al. (2012) Seronegative conversion after incomplete benznidazole treatment in chronic Chagas disease. Trans R Soc Trop Med Hyg 106: 636–638. doi: 10.1016/j.trstmh.2012.07.010 PMID: 22898619

30. Sguassero Y, Cuesta CB, Roberts KN, Hicks E, Comande D, et al. (2015) Course of Chronic Trypanosoma cruzi Infection after Treatment Based on Parasitological and Serological Tests: A Systematic Review of Follow-Up Studies. PLoS One 10: e0139363. doi: 10.1371/journal.pone.0139363 PMID: 26436678

31. Antunez MI, Cardoni RL (2001) Early IFN-gamma production is related to the presence of interleukin (IL)-18 and the absence of IL-13 in experimental Trypanosoma cruzi infections. Immunol Lett 79: 189–196. PMID: 11600197

32. Yunis JJ, Garcia O, Uriarte I, Yunis EJ (2000) Population data on 6 short tandem repeat loci in a sample of Caucasian-Mestizos from Colombia. Int J Legal Med 113: 175–178. PMID: 10876992
39. Sanchez-Diz P, Acosta MA, Fonseca D, Fernandez M, Gomez Y, et al. (2009) Population data on 15 autosomal STRs in a sample from Colombia. Forensic Sci Int Genet 3: e81–e82. doi:10.1016/j.fsigen.2008.08.002 PMID: 19414157

40. Ruiz-Linares A, Adhikari K, Acuna-Alonzo V, Quinto-Sanchez M, Jaramillo C, et al. (2014) Admixture in Latin America: geographic structure, phenotypic diversity and self-perception of ancestry based on 7,342 individuals. PLoS Genet 10: e1004572. doi:10.1371/journal.pgen.1004572 PMID: 25254375

41. Zingales B, Miles MA, Campbell DA, Tibayrenc M, Macedo AM, et al. (2012) The revised Trypanosoma cruzi subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol 12: 240–253. doi:10.1016/j.meegid.2011.12.009 PMID: 22226704

42. Moravej A, Rasouli M, Asael S, Kalani M, Mansoori Y (2013) Association of interleukin-18 gene variants with susceptibility to visceral leishmaniasis in Iranian population. Mol Biol Rep 40: 4009–4014. doi: 10.1007/s11033-012-2479-x PMID: 23269628

43. Anyona SB, Kempaiah P, Raballah E, Ouma C, Were T, et al. (2011) Functional promoter haplotypes of interleukin-18 condition susceptibility to severe malarial anemia and childhood mortality. Infect Immun 79: 4923–4932. doi: 10.1128/IAI.05601-11 PMID: 21969001

44. An P, Thio CL, Kirk GD, Donfield S, Goedert JJ, et al. (2008) Regulatory polymorphisms in the interleukin-18 promoter are associated with hepatitis C virus clearance. J Infect Dis 198: 1159–1165. doi: 10.1086/592047 PMID: 18781864

45. Cheong JY, Cho SW, Oh B, Kimm K, Lee KM, et al. (2010) Association of interleukin-18 gene polymorphisms with hepatitis B virus clearance. Dig Dis Sci 55: 1113–1119. doi: 10.1007/s10620-009-0819-z PMID: 19466545

46. Yue M, Wang JJ, Tang SD, Feng L, Zhang Y, et al. (2013) Association of interleukin-18 gene polymorphisms with the outcomes of hepatitis C virus infection in high-risk Chinese Han population. Immunol Lett 154: 54–60. doi: 10.1016/j.imlet.2013.08.007 PMID: 23978570

47. Kim YS, Cheong JY, Cho SW, Lee KM, Hwang JC, et al. (2009) A functional SNP of the Interleukin-18 gene is associated with the presence of hepatocellular carcinoma in hepatitis B virus-infected patients. Dig Dis Sci 54: 2722–2728. doi: 10.1007/s10620-009-0970-6 PMID: 19757044

48. Migita K, Sawakami-Kobayashi K, Maeda Y, Nakao K, Kondoh S, et al. (2009) Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease. Transl Res 153: 91–96. doi: 10.1016/j.trsl.2008.11.008 PMID: 19138654