Association of L-Ficolin Levels and FCN2 Genotypes with Chronic Chagas Disease

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Abstract

Background: L-ficolin (encoded by FCN2) binds to acetylated sugar moieties of many pathogen, including Trypanosoma cruzi, promoting their phagocytosis and lysis by the complement system.

Methods: We investigated L-ficolin levels in 160 T. cruzi infected patients with chronic Chagas disease and 71 healthy individuals, and FCN2 polymorphisms (−986 G>A, −602 G>A, and −4 A>G in the promoter and A258S in exon 8) in 243 patients, being 88 indeterminate (asymptomatic), 96 with cardiac, 23 with digestive and 33 with cardiodeg in manifestations (two were unspecified) and 305 controls (135 for A258S).

Results: Patients presented lower L-ficolin plasma levels than controls (p<0.0001). Among the different groups of cardiac commitment, individuals with moderate forms had higher L-ficolin levels than the severe forms (P = 0.039). Lower L-ficolin levels were found associated with the 258S variant in the patients (P = 0.034). We found less −4A/G heterozygotes in the cardiac patients, than in the controls (OR = 0.56 [95% CI = 0.33–0.94], P = 0.034). Heterozygote −4A/G genotypes with the 258S variant and 258SS homozygotes were nevertheless more frequent among cardiodeg patients than in controls (OR = 14.1 [95% CI = 3.5–56.8], P = 0.0001) and in indeterminate patients (OR = 3.2 [95% CI = 1.1–9.4], P = 0.037). We also found an association of the alleric frequency of the 258S variant with cardiodeg Chagas disease compared to controls (OR = 2.24 [95% CI = 1.1–4.5], P = 0.037). Thus, decreased patient levels of L-ficolin reflect not only protein consumption due to the disease process, but also the higher frequency of the 258S variant in patients with cardiodeg symptoms.

Conclusion: The very first study on Brazilian cohort associates both L-ficolin plasma levels and FCN2 variants to Chagas disease and subsequent disease progression. The prognostic value of L-ficolin levels and the FCN2*A258S polymorphism should be further evaluated in other settings.

Introduction

Chagas disease (CD) occurs in 18 different countries, mostly throughout South and Central America, and affects approximately 15 million people worldwide [1]. CD pathogenesis, caused by the flagellated protozoan Trypanosoma cruzi, is still poorly understood and there is no available marker that indicates the progression to the different clinical forms or prognosis of chronic disease. Despite the fact that approximately 50% of the individuals infected by T. cruzi stay in the indeterminate or asymptomatic form throughout their lives, which in general present good prognosis, each year about 2–5% of them progress to symptomatic forms of the disease, presenting irreversible cardiac and/or digestive and/or disorders [2]. A plausible presumption is that individuals who remain asymptomatic are able to reduce parasite numbers in the early phase of infection, and down modulate the immune response, limiting the development of pathology. On the other hand, individuals who will develop cardiac disease, although capable of parasite control, may not be capable of mounting efficient immunoregulatory mechanisms, thus leading to establishment of persistent inflammation [3].

The complement system has been shown to play a important role in the control of experimental T. cruzi infection, as well in clinical evolution of CD [1–8]. Ficolins are pattern-recognition proteins which bind to specific pathogen-associated molecular patterns (PAMP) on microorganism surfaces, promoting activation of the complement cascade through the lectin pathway thereby triggering the innate immune response [9]. Other putative functions of ficolins include binding to late apoptotic cells, apoptotic bodies and necrotic cells, enhancing their uptake by
macrophages [10]. The ficolins are synthesized as a single polypeptide containing N-collagen-tails and C-terminal fibrinogen-like binding domains, which are oligomerized into higher oligomeric forms [9]. In humans, three ficolin genes have been identified: FCN1, FCN2 and FCN3, which encode M-ficolin (ficolin-1), L-ficolin (ficolin-2) and H-ficolin (Hakata antigen or ficolin-3), respectively. Single nucleotide polymorphisms (SNPs) in the promoter region of the FCN2 gene have been associated with the variability in the individual serum concentrations of the protein. Studies have shown that the presence of the nucleotide adenine \(A\) at positions \(-986\ A>G\) and \(-602\ A>G\) as well as the nucleotide guanine \(G\) at the position \(-4G>A\) are related to higher L-ficolin serum levels [11]. In addition, two polymorphisms located in exon 8, encoding the fibrinogen-like domain (containing T236M and A258S) are associated with decreased and increased ability of binding to acetylated residues, respectively [11–13]. L-ficolin levels and FCN2 polymorphisms have been reported to be associated with different diseases [14–20].

Both L-ficolin and H-ficolin are able to interact with the mannan-binding lectin (MBL)-associated serine proteases, promoting activation of the complement cascade [21]. L-ficolin recognizes N-acetylated molecules, including N-acetylgalactosamine (GlcNAc) or N-acetylgalactosamine (GalNAc) [22,23]. The surface of \(T. cruzi\) contains a large diversity of N-linked and O-linked carbohydrate-rich molecules and it was recently demonstrated that \(T. cruzi\) activates the lectin pathway [7]. The authors have shown that a fast binding of L-ficolin occurs on the surface of \(T. cruzi\) and that the depletion of these molecules in the serum leads to failure of parasite clearance by the complement, indicating that the lectin pathway obviously plays an important role in host defense against this pathogen. Although it has been demonstrated that L-ficolin is involved in the host defense against \(T. cruzi\) infection, there are no studies on ficolins and chronic CD so far. In this study we aim to evaluate whether L-ficolin levels and FCN2 polymorphisms could be possible prognostic markers for susceptibility to the different clinical forms of CD.

### Materials and Methods

#### Ethics Statement

Formal written consent approved by the ethics committee was obtained from each individual. The project was approved by the ethics committee of Hospital De Clinicas, Universidade Federal do Paraná (CEP/HC-UFPR n.1457.122/2007-06).

#### Subjects and Samples

A total of 243 chronic CD patients attended at the Chagas Disease Ambulatory of the Clinical Hospital of the Federal University of Paraná (HC-UFPR) were investigated (Mean age 57.3 [34–90] years; 58% female, 42% male; 76.5% Euro-Brazilian, 18.9% Afro-Brazilian, 4.1% American, 0.5% Asian). CD diagnosis was performed by serological and clinical examinations. The clinical history of the patients was obtained from medical records and interviews, using a standard questionnaire. Patients younger than 18 years-old, with a history of blood transfusion, recent infections or suspected non-chagasic cardiomyopathy (such as hypertensive cardiopathy) were excluded. The cardiac patients were graded according to the cardiac insufficiency classification of the American Heart Association (AHA), adapted for Chagas disease [24]. Detailed demographic and clinical characteristics of the specific CD forms are given in Table 1. A group of 305 unrelated South Brazilians with negative Chagas (anti-\(Trypanosoma cruzi\)) serology and without non-chagasic cardiomyopathy were used as controls (Mean age 41 [18–75] years; 49.2% female, 50.8% male; 85.2% Euro-Brazilian, 12.8% Afro-Brazilian, 1.3% American, 0.7% Asian). Ethic background of patients and controls was determined as previously described [25].

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### Table 1. Demographic and clinical parameters of chronic Chagas patients.

| Parameters | Chagas form | Indeterminate | Cardiac | Digestive | Cardiodigestive |
|------------|-------------|---------------|---------|-----------|-----------------|
| N          | 88          | 96            | 23      | 33        |
| Age (years) |             |               |         |           |                 |
| Average    | 54.6        | 58.9          | 59.3    | 58.2      |
| [Min-Max]  | [34–76]     | [34–90]       | [36–84] | [37–73]   |
| Gender (%) |             |               |         |           |                 |
| Male       | 61.4        | 50.0          | 21.7    | 57.6      |
| Ethnic group (%) |           |               |         |           |                 |
| European   | 87.5        | 67.7          | 65.2    | 78.8      |
| African    | 9.1         | 26.0          | 30.4    | 18.2      |
| Asian      | 0           | 1.0           | 4.3     | 0         |
| Amerindian | 3.4         | 5.2           | 0       | 3         |
| Class of cardiac Commitment (%) | | | | | |
| A          | 3.4         | 24.0          | N.d.    | 33.3      |
| B          | 23.0        | 25.0          | N.d.    | 21.2      |
| C          | 1.1         | 36.4          | N.d.    | 33.3      |
| D          | 0           | 20.8          | N.d.    | 6.1       |
| Left ventricular ejection fraction (%) | | | | | |
| N          | [47–132.8]  | [35–78]       | [79–58] | [24–84]   | N.d.            |
| B          | 78.9        | 67.7          | 65.2    | 78.8      |
| C          | 9.1         | 26.0          | 30.4    | 18.2      |
| D          | 0           | 1.0           | 4.3     | 0         |
| C-reactive protein* mg/dl | | | | | |
| N          | [28–0.74]   | [0.08–3.77]   | [47–0.66] | [0.08–4.25] | [0.19 [0.09–0.31] |
| B          | 0.74        | 0.66          | 0.19    | 0.33      |
| C          | 0.19        | 0.08          | 0.76    | 0         |
| D          | 0           | 0             | 0       | 0         |
| Mannose-binding lectin* ng/dl | | | | | |
| N          | [53–3514]   | [42–6379]     | [58–2270] | [50–7214] | [1909 [50–5465] |
| B          | 3514        | 2270          | 7214    | 5465      |
| C          | 1909        | 2222          | 5465    | 5600      |
| D          | 0           | 0             | 0       | 0         |

*Mannose-binding lectin* levels were determined by ELISA and C-reactive protein (hs-CRP) by nephelometry as previously described [6] n number of investigated samples; N.d. not determined.

*some patients were unspecified.

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After signing the informed formal written consent, three ml of venous blood from each individual was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA). Samples were maintained on ice after collection and during transportation to the laboratory. After centrifugation at $-4^\circ$C plasma separation from whole blood was performed as quickly as possible, samples were aliquoted on ice and immediately stored at $-80^\circ$C until used. Genomic DNA was extracted from peripheral whole blood using commercial kits (GFX™ Genomic Blood DNA Purification Kit, GE Healthcare, São Paulo, Brazil), according to the manufacturer’s instructions.

L-ficolin Measurement

The quantification of plasma L-ficolin levels was performed in 71 controls (of which 62 were genotyped) and 160 patients (of which 158 were genotyped, being 54 indeterminate, 54 with some kind of cardiomyopathy, 19 with digestive symptoms, 29 with the cardiodiagnostic form and two unspecified). L-ficolin levels were determined using a commercially available enzyme linked immunosorbent assay (ELISA) kit (HK396, Hyclon® Biotech, Uden, Netherlands), according to the manufacturer’s instructions. The detecting range of this kit is 16–1000 ng/ml of the protein.

Genotyping of FCN2 Variants

Three promoter SNPs including $-986\ G>A$ (rs3124952), $-602\ G>A$ (rs1324953), $-4\ A>G$ (rs17514136) and $A258S$ in exon 8 (rs7851696) were genotyped in the patients using Fluorescence Resonance Energy Transfer (FRET) based real-time PCR assay as previously described [26], and in 135 controls through sequencing [14]. Additionally, 170 patients were genotyped for the SNPs $-986\ G>A$ (rs3124952), $-602\ G>A$ (rs1324953) and $-4\ A>G$ (rs17514136) using PCR amplification with sequence-specific primers (SSP) (Table 2). In order to reconstruct the haplotypes, we performed three reactions called FCN2 Prom12, FCN2 Prom23 and FCN2Prom13, using the primers listed in table 2. In order to validate the reactions, we added two generic primers, either HGHf and HGHr or FCN2Ex8f and FCN2Ex8r. PCR amplifications were carried out in a Therm-2000 (Axygen, United States) in a final volume of 15 $\mu$l (Invitrogen Life Technologies or Promega, United States), 0.35–0.7 mM of Q solution (Invitrogen Life Technologies, Netherlands), 2 mM of MgCl$_2$ (Invitrogen Life Technologies, United States), 1X Coral Load PCR buffer (Qiagen, The Netherlands), according to the manufacturer’s instructions. The PCR protocol was followed by 30 cycles of annealing temperatures every 10 cycles was performed, using 68 $^\circ$C-64 $^\circ$C-62 $^\circ$C for FCN2 Prom12, 65 $^\circ$C-62 $^\circ$C-59 $^\circ$C for FCN2 Prom23 and 69 $^\circ$C-67 $^\circ$C-65 $^\circ$C for FCN2Prom13. According to a previous study, this strategy assures higher specificity to the amplification and provides a larger amount of the desired PCR product [27]. The promoter haplotypes amplified by a pair of SSPs were identified by the presence or absence of specific bands when run on a 1.2% agarose gel electrophoresis. Positive controls reassured the quality of the reactions.

Statistical Analysis

Statistics was done using the statistical package for social sciences (SPSS) version 10.0, STATA ver 9.1 and with the Graphpad Prism 5.04 software package. Data were normalized and have been analyzed by STATA. Genotype and allele frequencies were obtained by direct counting. The hypothesis of Hardy-Weinberg equilibrium and of homogeneity between genotype distributions (exact test of population differentiation of Raymond and Rousset) were executed with the ARLEQUIN software package version 3 [28]. Possible associations between FCN2 genotypes/haplotypes/variants and different clinical forms were analyzed with two tailed Fisher’s exact test. Additionally multivariate analysis was executed to validate whether variables such as age, gender and ethnicity may possibly influence the clinical outcome by MANOVA. L-ficolin concentrations in the different groups were presented by the median and range and compared between the genotypes using either nonparametric Mann-Whitney or Kruskal-Wallis tests. Also correlation between L-ficolin levels to age was tested using spearman’s rank correlation. Unless otherwise stated, two-tailed P-values less than 5% were considered significant. P-values for correlations are provided after correction for false detection rate (FDR).

Results

L-ficolin Levels

Higher L-ficolin plasma concentrations were observed in the controls when compared to Chagas patients (median: 4252 ng/ml vs. 2538 ng/ml, P<0.0001). Similar trend was observed between indeterminate and symptomatic patients (median: 2888 ng/ml vs. 2519 ng/ml, one-tailed P = 0.051, two-tailed P = 0.10) (Figure 1). No significant correlation between L-ficolin levels and age has been observed using Spearman’s rank correlation test (Spearman’s rho = -0.1402, P = 0.08). Nevertheless L-ficolin levels were higher in the group with 45 to 59 years of age, compared with those aged above 60 years (median: 2834 ng/ml vs. 2265 ng/ml, P = 0.005). Nevertheless they were still lower than the levels found in the 45–59 years group of controls, which had a median of 4311 ng/ml (P = 0.0007) (Figure 2). There was also a trend to increased L-ficolin concentration in the healthy adults (P = 0.072). Among the different groups of cardiac commitment, individuals with the less severe B form had higher L-ficolin levels than the C and D forms (median: 2981 ng/ml vs. 2094 ng/ml, P = 0.039) (Figure 2). Among the clinical parameters (listed in Table 1), only mannose-binding lectin correlated weakly with L-ficolin levels in the indeterminate patients (R2 = 0.38, P = 0.004), but not in the other disease stages (not shown). Tests for multivariate analysis revealed no significant effects of age, gender or ethnicity to L-ficolin levels.

FCN2 Polymorphisms

Five haplotypes comprehending the $-986\ G>A, -602\ G>A, -4\ A>G$ variants in the promoter and the amino acid substitution $A258S$ in exon 8 were identified: $AAAA, AGGA, AGGA, GAGA$ and $GGGS$ (Table 3). Haplotypes distributions did not differ between the different group and genotype distributions were in Hardy Weinberg equilibrium. Nevertheless we observed less $4A/G$ heterozygotes (comprehending the promoter genotypes $AAAA, AGGA/AGGA$ and $AGGA/GGGA$ in the cardiac patients than in the controls (23/96 or 24% vs. 110/305 or 36.1%, OR = 0.56 [95% CI = 0.33–0.94], P = 0.034), and there was also a trend in the same direction between cardiac and indeterminate patients (P = 0.077) (Table 3). Heterozygote $4A/G$ genotypes with the $258S$ variant in exon 8 ($AGGA/GGGA$ and $258S$ homozygotes ($GGGS/GGGS$) were more frequent among cardiodigestive patients, than in controls (8/33 or 24.2% vs. 3/135 or 2.22%, respectively, OR = 14.1 [95% CI = 3.5–56.8], P = 0.0001) and in indeterminate patients (8/33 or 24.2% vs. 8/88 or 9.1%, OR = 3.2 [95% CI = 1.1–9.8], P = 0.03).
We also found an association of the allelic frequency of the 258S variant with cardiodigestive Chagas disease (14/66 or 21.2% vs. 29/270 or 10.7% in the controls, OR = 2.24 [95%CI = 1.1–4.5], P = 0.037) (Tables 3 and 4).

Association of L-ficolin Levels with FCN2 Genotypes

Lower L-ficolin levels were found associated with the 258S variant in the patients (median: 2419 ng/ml vs. 2748 ng/ml in genotypes without 258S, P = 0.034). There was no difference

Table 2. Primers used in the sequence-specific PCR (PCR-SSP) for the –986, –602 and –4 promoter FCN2 polymorphisms.

| Forward Primer | Reverse primer | size |
|----------------|----------------|------|
| PCR-SSP Prom1,2 | FCN2 Prom –986 Af 5’ ACCTCGGCATCCCGATGGCA 3’ FCN2 Prom –602 Ar 5’ TATGTAGAGCAGGGGACAC 3’ | 374 bp |
| PCR-SSP Prom1,2 | FCN2 Prom –986 Gf 5’ ACCTCGGCATCCCGATGGCG 3’ FCN2 Prom –602 Gr 5’ TATGTAGAGCAGGGGACAC 3’ | 374 bp |
| PCR-SSP Prom2,3 | FCN2 Prom –602 Af 5’ TCTCTCTTTCTCTCGTTCA 3’ FCN2 Prom –4 Ar 5’ GCTCTGTCCAGCTCATCTCT 3’ | 648 bp |
| PCR-SSP Prom2,3 | FCN2 Prom –602 Gf 5’ TCTCTCTTTCTCTCGTTCA 3’ FCN2 Prom –4 Gr 5’ GCTCTGTCCAGCTCATCTCC 3’ | 648 bp |
| Endogenous control | HGH f 5’ TGCCCTCCAACCATTCCCTTA 3’ HGH r 5’ CCACTCAGGGTATTCTGGTGTTC 3’ | 431 bp |
| Endogenous control | FCN2 Ex8f 5’ GCCAGGCTCTCACATATAAG 3’ FCN2 Ex8r 5’ AAAGGGTGTGATGGGGGAAAC 3’ | 500 bp |
| Primer design was based on the NT_019501 reference sequence. FCN2 – Ficolin 2; HGH – Human Growth Hormone; f – forward; r – reverse; In bold: variant nucleotides; bp – base pairs; Prom – promoter; Ex8 – exon 8. doi:10.1371/journal.pone.0060237.t002

Figure 1. L-ficolin levels in Chagas patients and controls. The statistical distribution is shown with median (line in the box), box indicating the 25–75 percentiles, whiskers the 5–95 percentile and arithmetic mean (cross inside the box).

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between the levels according to the other genotypes, even in the controls (not shown). Whereas heterozygote genotypes with \(24A/G\) were, in general, widely distributed according to L-ficolin levels in the different groups, those with \(258S\) (genotype \(AGGA/GGAS\)) as well as \(258S/S\) (\(GGAS/GGAS\)) homozygotes, clearly associated with concentrations under 3000 ng/ml (Wilcoxon signed rank test, \(P = 0.04\)) (Figure 3).

### Discussion

In this study, L-ficolin levels were measured in plasma of the Chagas patients and controls. L-ficolin levels in the controls were within the range reported for healthy European adults in the early studies [29,30]. A decreased L-ficolin plasma level were observed in the Chagas patients when compared to controls, and a similar trend was observed between symptomatic patients and indeterminate, however, this difference was not significant. Importantly,

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**Table 3. FCN2 allele and haplotype frequencies (± standard deviation) in Chagas patients and controls.**

| FCN2     | Controls 610 | Patients 486 | Indeterminate 176 | Symptomatic 304 | Cardiac 192 | Digestive 46 | Associated 66 |
|----------|--------------|--------------|-------------------|-----------------|-------------|--------------|---------------|
| \(-986G>A\) | 50.0±2.5     | 43.4±2.3     | 46.0±3.8          | 41.4±2.8        | 40.6±3.6    | 43.5±7.4     | 42.4±6.1       |
| \(-602G>A\) | 19.3±2.0     | 17.9±1.7     | 19.3±3.0          | 16.8±2.1        | 16.7±2.7    | 15.2±5.4     | 18.2±4.8       |
| \(-4A>G\) | 23.4±2.2     | 20.6±1.8     | 20.5±3.0          | 20.7±2.3        | 19.3±2.9    | 23.9±6.4     | 22.7±5.2       |
| A258S    | 10.7±1.9*    | 15.2±1.6     | 13.6±2.6          | 16.1±2.1        | 14.6±2.6    | 15.2±5.4     | 21.2±5.1       |
| AAAA     | 19.3±2.0     | 17.9±1.7     | 19.3±3.0          | 16.8±2.1        | 16.7±2.7    | 15.2±5.3     | 18.2±4.8       |
| AGGA     | 23.4±2.2     | 20.6±1.8     | 20.5±3.0          | 20.7±2.3        | 19.3±2.9    | 23.9±6.4     | 22.7±5.2       |
| AGAA     | 7.3±1.3      | 4.9±1.0      | 6.3±1.8           | 3.9±1.1         | 4.7±1.5     | 4.3±3.0      | 1.5±1.5        |
| GGA      | 52.0±2.0     | 56.6±2.3     | 54.0±3.8          | 58.6±2.8        | 59.4±3.6    | 56.5±7.4     | 57.6±6.1       |
| GGAA     | 41.1±3.0     | 41.4±2.2     | 40.3±3.7          | 42.4±2.8        | 44.8±3.6    | 41.3±7.3     | 36.4±6.0       |
| GGAS     | 10.7±1.9*    | 15.2±1.6     | 13.6±2.6          | 16.1±2.1        | 14.6±2.6    | 15.2±5.4     | 21.2±5.1       |

*In bold: significant difference (see text).

*Note:* A258S was investigated by sequencing in a subset of 135 samples. N number of chromosomes.

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Figure 2. Distribution of L-ficolin levels based on age and clinical classification. (A) L-ficolin levels in Chagas patients and controls according to age (B) L-ficolin levels in Chagas patients and controls based on class of cardiac commitment. The statistical distribution is shown with median and interquartile range. One outlier (18880.7 ng/ml) was excluded for better visualization in the 45–59 group of patients.

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serum L-ficolin concentrations were also lower in Bronchiectasis patients than controls from the UK [29]. In contrast to our previous work with the same patients, where MBL levels increased gradually according to age, L-ficolin levels were higher in the group with 45 to 59 years of age, compared with those aged above 60 years. There was also a trend to increased L-ficolin concentration in the healthy adults (P = 0.072), but others did not find any relationship between L-ficolin concentration and age in healthy Scottish adults [29]. There was no significant difference between L-ficolin levels according to the gender of patients and controls, as expected [29]. In addition, the difference of L-ficolin levels was observed among the different groups of cardiac commitment. Of which individuals with the less severe B form had higher L-ficolin levels than the C and D forms. Interestingly, median L-ficolin levels are higher in acute than in chronic hepatitis B conditions [15]. They are also higher in acute malaria, compared with the levels after treatment [16], but lower in Schistosoma haematobium-infected individuals from Nigeria, compared to controls [17]. Our results contrast with those found by our group in the same patients regarding MBL levels, which were much higher (above 1000 ng/ml) in patients with chagasic cardiomyopathy and did not differ between the B C and D stages [6]. Furthermore, a weak correlation between mannose-binding lectin and L-ficolin levels was observed in the indeterminate patients, but not in the other disease stages.

Allele and haplotype frequencies in the controls did not differ from those reported for European-derived populations [11,26]. No significant difference of haplotype distribution was observed between different groups, nevertheless, −4 A/G heterozygotes was observed to associate with the disease when compared cardiac patients to the controls as well as cardiac to the indeterminate patients. In addition, the AGA promoter haplotype, whose presence is obligatory in −4 A/G heterozygotes, was also associated with protection against HBV infection in Vietnamese [15], but with susceptibility to schistosomiasis in Nigeria [17]. Our group found the AGA haplotype to be associated with protection against clinical leprosy and rheumatic fever [14,18], whereas others found it associated with susceptibility to cutaneous leishmaniasis [28]. Ficolin-2 seems to be, as MBL, a double-edged sword in immunity, explaining the contrasting results in association studies with different infectious diseases. We also found an association of the allelic frequency of the 258S variant with cardiodigestive Chagas disease (Tables 3 and 4). The 258S variant was also found associated by others with earlier onset of Pseudomonas aeruginosa colonization in cystic fibrosis patients [31]. In contrast to our findings, homozygosity for this variant was associated with protection against cutaneous leishmaniasis in Syria [32] and the presence of this allele, with protection against cytomegalovirus, but not bacterial infections, after orthotopic liver transplantation [33,34]. It was not associated with respiratory tract infections in Dutch children [35], nor with invasive pneumococcal disease [36], rheumatoid arthritis [37] and Behçet’s disease in Japan [38]. Interestingly, FCN2*258S, also known as FCN2-C, has increased N-acetyl-D-glucosamine (GlcNAc) binding capacity [11].

Figure 3. Distribution of L-ficolin levels based on genotypic variant and disease stages. (A) L-ficolin levels in Chagas patients and controls by the presence of 258S variant and (B) Distribution of L-ficolin levels in Chagas patients and controls by disease stages. The statistical distribution is shown with median and interquartile range. Closed dots: samples with −4 A/G genotype. Closed boxes: samples with −4 A/G and 258A/S (AGGA/GGAS genotype) or 258S/S homozygotes (GGAS/GGAS genotype).

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and O-linked GlcNAc moieties constitute a common epitope between cruzipain, a major T. cruzi antigen, and either myosin or other cardiac O-GlcNAc-containing proteins [39]. Higher GlcNAc binding capacity of this variant may thus correlate with the disease process itself, since L-ficolin can bind to T. cruzi antigens [7], to pentraxin 3 [41] and to the C-reactive protein [42], which might affect circulating levels. In addition, inflammatory activity and the resulting tissue fibrosis could be further exacerbated by the higher capacity of GlcNAc binding to the 258S variant. L-ficolin levels and consumption could thus be used as markers of disease activity, but further experiments should be performed to prove this hypothesis.

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Author Contributions

Conceived and designed the experiments: IJTMR TPV JFJK. Performed the experiments: PRL ABWB CG. Analyzed the data: ABWB TPV PRL. Contributed reagents/materials/analysis tools: TPV IJTMR. Wrote the paper: PRL ABWB TPV IJTMR.

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Table 4. FCN2 genotype frequencies (%) in Chagas patients and controls.

| FCN2 N | Controls (135) | Patients (248) | Indeterminate (88) | Symptomatic (152) | Cardiac (96) | Digestive (23) | Associated (33) |
|--------|----------------|----------------|-------------------|-------------------|-------------|--------------|----------------|
| AAAA/AAAA | 10 (3.3) | 6 (2.5) | 3 (3.4) | 3 (2.0) | 2 (2.1) | 0 | 1 (3.0) |
| AAAA/AGAA | 6 (2.0) | 7 (2.9) | 3 (3.4) | 4 (2.6) | 3 (3.1) | 0 | 1 (3.0) |
| AAAA/GGGA | 22 (7.2) | 14 (5.8) | 7 (8.0) | 6 (4.0) | 3 (3.1) | 2 (8.7) | 1 (3.0) |
| AAAA/GGAA | 20 (14.8)* | 36 (14.8) | 11 (12.5) | 25 (16.4) | 15 (15.6) | 4 (17.4) | 6 (18.2) |
| AAAA/GGAs | 7 (5.2)* | 18 (7.4) | 7 (8.0) | 10 (6.6) | 7 (7.3) | 1 (4.3) | 2 (6.1) |
| AAAA/GGA | 57 (18.7) | 54 (22.2) | 18 (20.5) | 35 (23.0) | 22 (22.9) | 5 (21.7) | 8 (24.2) |
| AGAA/AGAA | 0 | 1 (0.4) | 1 (1.1) | 0 | 0 | 0 | 0 |
| AGAA/AGGA | 9 (3.0) | 4 (1.6) | 3 (3.4) | 1 (0.7) | 1 (1.0) | 0 | 0 |
| AGAA/GGAA | 7 (5.2)* | 9 (3.7) | 1 (1.1) | 7 (4.6) | 5 (5.2) | 2 (8.7) | 0 |
| AGAA/GGAs | 2 (1.5)* | 2 (0.8) | 2 (2.3) | 0 | 0 | 0 | 0 |
| AGAA/GGA | 27 (8.9) | 11 (4.5) | 3 (3.4) | 7 (4.6) | 5 (5.2) | 2 (8.7) | 0 |
| AGGA/AGGA | 18 (5.9) | 12 (4.9) | 2 (2.3) | 10 (6.6) | 7 (7.3) | 2 (8.7) | 1 (3.0) |
| AGGA/GGAA | 31 (23.0)* | 36 (14.8) | 15 (17.0) | 21 (13.8) | 12 (12.5) | 3 (13.0) | 6 (18.2) |
| AGGA/GGAS | 3 (2.2)* | 22 (9.1) | 7 (8.0) | 15 (9.9) | 7 (7.3) | 2 (8.7) | 6 (18.2) |
| AGG/GGA | 79 (25.9) | 58 (23.9) | 22 (25.0) | 36 (23.7) | 19 (19.8) | 5 (21.7) | 12 (36.4) |
| GGAA/GGAA | 18 (13.3)* | 47 (19.3) | 19 (21.6) | 28 (18.4) | 20 (20.8) | 3 (13.0) | 5 (15.2) |
| GGAA/GGAs | 17 (12.6)* | 26 (10.7) | 6 (6.8) | 20 (13.2) | 14 (14.6) | 4 (17.4) | 2 (6.1) |
| GGAs/GGAs | 0* | 3 (1.2) | 1 (1.1) | 2 (1.3) | 0 | 0 | 2 (6.1) |
| GGA/GGA | 77 (25.2) | 76 (31.3) | 26 (29.5) | 50 (32.9) | 34 (35.4) | 7 (30.4) | 9 (27.3) |

In bold: genotypes with ~4 A/G heterozygosity, whose summed frequencies differ between patients and controls. Gray-shadowed: genotypes AGGA/GGAs and GGGA/GGAs (homozygote for A258S), whose summed frequencies differ between indeterminate patients and controls (see text). *A258S was investigated by sequencing in a subset of 135 controls. n number of individuals.

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and other proteins involved in the innate immune response. The complement system plays a crucial role in the defense against pathogenic parasites, and it has been shown to be activated in Chagas disease [7]. L-ficolin is a component of the complement system and has been implicated in the clearance of pathogens [8]. In our study, we investigated the association between L-ficolin levels and Chagas disease. We observed a decreased frequency of the A258S variant in patients compared to controls. This variant has been associated with increased GlcNAc binding capacity, which may affect the function of L-ficolin in the disease process.

Table 4 presents the genotype frequencies of the FCN2 gene in Chagas patients and controls. The table shows a significant association between the A258S variant and Chagas disease, with a lower frequency of this variant in patients compared to controls. This finding suggests that the A258S variant may be protective against the disease.

In conclusion, the study provides evidence for a genetic association between the A258S variant of the FCN2 gene and Chagas disease, indicating a potential role for L-ficolin in the disease pathogenesis. Further research is needed to elucidate the mechanisms underlying this association and to explore the clinical implications of these findings.
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