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Short Report: Mannose-Binding Lectin and Toll-Like Receptor Polymorphisms and Chagas Disease in Chile

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Abstract. Mannose-binding lectin (MBL) and Toll-like receptor (TLR) polymorphisms may influence susceptibility and manifestation of *Trypanosoma cruzi* infection. In northern Chile, we examined 61 asymptomatic patients with chronic Chagas disease (CD), 64 patients with chronic Chagas cardiomyopathy (CCC), and 45 healthy individuals. Low-producer *MBL2*B* genotypes were more common in CD patients (48%) than healthy individuals (31%; adjusted odds ratio = 2.3, 95% confidence interval = 1.01–5.4, $P = 0.047$) but did not differ with manifestation. In contrast, the heterozygous *Toll-like receptor 4* (*TLR4*)-deficiency genotype D299G/T399I occurred more frequently in asymptomatic (14.8%) than CCC patients (3.1%; $P = 0.02$). *TLR1*-I602S, *TLR2*-R753Q, *TLR6*-S249P, and *MAL/TIRAP*-S180L did not associate with CD or CCC. These findings support the complement system to be involved in defense against *Trypanosoma cruzi* infection and indicate that curbed TLR4 activation might be beneficial in preventing CCC.

Chagas disease (CD), caused by *Trypanosoma cruzi*, affects up to 8 million people in Latin America, with up to 13,000 annual deaths. Infection persists lifelong. Still, only one-third of patients develop manifestations, mainly chronic Chagas cardiomyopathy (CCC). The pathogenesis of progression to chronic symptomatic CD is poorly understood, but it involves immunological and autoimmunological factors and genetic disposition.[1,2] Innate immune responses contribute to host control, and *T. cruzi* has been shown to bind to mannose-binding lectin (MBL), which activates the lectin pathway of early complement killing and reduces host cell invasion.[3] Also, *T. cruzi* activates the Toll-like receptor (TLR) system, particularly TLRs 2 and 4, which ultimately leads to a proinflammatory response.[4]

Genetic variation of innate immunity may influence infection and manifestation, which has been shown for *MBL* and *TLR* single-nucleotide polymorphisms (SNPs) and various infectious and inflammatory diseases.[5,6] Specifically, an increased risk of severe CCC has been attributed to high MBL serum levels,[7] but respective genotypic data are not available. In contrast, a mutation in the TLR adaptor protein MAL/TIRAP (S180L) has been associated with a reduced risk of CCC.[8]

In the provinces of Choapa and Limarí, northern Chile, CD is highly endemic, and although Chile was declared free of domestic *T. cruzi* transmission in 1999, vertical transmission and management of chronically infected patients continue.[9] In this area, a cohort of patients has been regularly followed-up since the 1990s.[10] In these patients and healthy individuals, we typed common SNPs that reduce the function of TLRs involved in the recognition of *T. cruzi* as well as low-producer *MBL2* alleles and analyzed associations with CD and CCC.

Patients were recruited from the CD cohort in November of 2007 and May of 2008. In the patients' villages, healthy volunteers were asked to participate as controls. All study participants were thoroughly informed about study purpose and procedures, and they signed an informed consent. The study was approved by the ethics committee of the medical faculty at the Universidad de Chile, Santiago, Chile.

CD patients were defined by a positive enzyme immunoassay (EIA) test using *T. cruzi*-specific antigens and confirmed by an indirect immunofluorescence test (IFT).[11] Parasitemia was assessed by xenodiagnosis and polymerase chain reaction (PCR).[12,13] Controls were defined by a negative *T. cruzi* EIA.

All CD patients were examined by 12-lead electrocardiography (ECG), and ECG strips were evaluated by an experienced cardiologist who was blinded to patient status. For the diagnosis of CCC, World Health Organization criteria were applied.[14]

Blood samples were collected into ethylenediaminetetraacetic acid (EDTA), and genomic DNA was extracted (QIAmp DNA Blood Mini Kit; Qiagen, Hilden, Germany). Fluorescence-labeled hybridization detected *TLR1*-I602S, *TLR2*-R753Q, *TLR4*-D299G, and *TLR4*-T399I.[15,16] For *TLR6*-S249P, primers GAAAGACTCTGACCAGGCAT and CTAGTTTATTCG CTATCCAAGTG along with probes ACCAGAGGTCCAA CCTTACTGAA-FL and LCRed640-TTACCCTCAACCAC ATAGAAACGACTTGGA were used. *MAL/TIRAP*-S180L was identified by a high-resolution melting dye assay using published primers.[8] Polymorphisms associated with lowered MBL concentration or function were typed by PCR-restriction fragment length polymorphism, including the functional mutations *MBL2*B* (G54D), *MBL2*C* (G57E), and *MBL2*D* (R52C) as well as the −221 (X/Y) promoter polymorphism.[17,18]

Data were analyzed with Statview 5.0 (SAS Institute Inc., Cary, NC). Genotypes (heterozygous, homozygous, and/or combined) and allele frequencies were compared by χ^2 or Fisher exact test. Associations of genotypes with CD or CCC were expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs), and analyzes were adjusted for age and residence by logistic regression as indicated to produce adjusted ORs (aORs). A P value < 0.05 was considered statistically significant.

Individuals (172) were recruited, including 127 CD patients. Two patients were excluded because of unsuccessful SNP typing. Of the remaining patients, 61 were asymptomatic,

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and 64 had CCC. *T. cruzi* parasitemia was detected by xenodiagnosis in 11.4% (14/123) and PCR in 43.5% (50/115) of patients without differences between patient subgroups. Patients were older than healthy individuals, and those patients with CCC were older than asymptomatically infected subjects. Also, residence differed between controls and patients (Table 1). SNPs were in Hardy–Weinberg equilibrium in all subgroups except for *TLR6*-S249P in CD patients ($P = 0.008$).

*MBL2*C* and *MBL2*D* were absent in the study population, but *MBL2*B* (heterozygous and homozygous combined) was common at 43.5%. *MBL2*B* was seen in 31% of healthy individuals and 48% of CD patients (OR = 2.01, 95% CI = 0.9–4.5, $P = 0.05$). Correspondingly, the allele frequency was higher in patients ($P = 0.04$). Adjusting for differences in age (years) and residence between healthy subjects and patients, *MBL2*B* (heterozygous and homozygous combined) was associated with increased odds of CD (aOR = 2.3, 95% CI = 1.01–5.4, $P = 0.047$). No differences in *MBL2*B* between asymptomatic and CCC patients were seen (Table 1). As for the −221 (X/Y) promoter SNP, no association with CD or CCC was discernible.

The heterozygous *TLR4*-D299G/T399I genotype (complete cosegregation) occurred in 8% of the study population and did not differ between healthy individuals and CD patients. However, it occurred more frequently in asymptomatically infected subjects than CCC patients (OR = 5.4, 95% CI = 1.03–52.6, $P = 0.02$). Adjusted for age, this estimate was slightly reduced (aOR = 4.6, 95% CI = 0.9–23.0, $P = 0.06$). *TLR1*-I602S, *TLR2*-R753Q, and *TLR6*-S249P did not show evidence of association with CD or CCC. *MAL*-S180L genotypes (heterozygous and homozygous) only tended to be more common in healthy individuals (26.7%) than CD patients (17.6%; $P = 0.19$), and also, variant allele frequencies ($P = 0.07$) tended to be more common in healthy individuals.

Susceptibility to *T. cruzi* infection and manifestation are subject to genetic disposition, which has been estimated to account for more than one-half of the observed variation in infection status.[2]

TABLE 1
Genotypes and allele frequencies in healthy individuals and patients with Chagas disease

Parameter	Healthy	Chagas disease		
		All	Asymptomatic	Cardiomyopathy
Number	45	125	61	64
Median age in years (range)	29 (1–87)	45 (2–74)*	40 (2–74)*	48.5 (26–70)*†
Female (%)	80.0	77.6	83.6	71.9
Residence (%)				
Combarbala ($N = 36$)	24.4	20.0	18.0	21.9
Illapel ($N = 37$)	6.7	27.2	23.0	31.3
Canela ($N = 31$)	40.0	10.4	13.1	7.8
Salamanca ($N = 25$)	8.9	16.8	23.0	10.9
Others ($N = 41$)	20.0	25.6*	23.0*	28.1*
*MBL2*B* (%)				
Wild type	31 (68.9)	65 (52.0)	31 (50.8)	34 (53.1)
Heterozygous	13 (28.9)	51 (40.8)	25 (41.0)	26 (40.6)
Homozygous	1 (2.2)	9 (7.2)	8.2 (5)	4 (6.3)
Variant allele frequency	0.167	0.276*	0.287*	0.266
MBL2 −221 promoter (%)				
YY	34 (75.6)	101 (80.8)	50 (82.0)	51 (79.7)
YX	11 (24.4)	22 (17.6)	11 (18.0)	11 (17.2)
XX	0	2 (1.6)	0	2 (3.1)
Variant allele (X) frequency	0.122	0.096	0.090	0.117
TLR1-I602S (%)				
Wild type	27 (60.0)	84 (67.2)	40 (65.6)	44 (68.8)
Heterozygous	16 (35.6)	31 (24.8)	15 (24.6)	16 (25.0)
Homozygous	2 (4.4)	10 (8.0)	6 (9.8)	4 (6.3)
Variant allele frequency	0.222	0.204	0.221	0.188
TLR2-R753Q (%)				
Wild type	44 (97.8)	123 (98.4)	100	62 (96.9)
Heterozygous	1 (2.2)	2 (1.6)	0	2 (3.1)
Variant allele frequency	0.011	0.008	0	0.016
TLR4-D299G (%)				
Wild type	42 (93.3)	114 (91.2)	52 (85.2)	62 (96.9)
Heterozygous	3 (6.7)	11 (8.8)	9 (14.8)	2 (3.1)†
Variant allele frequency	0.033	0.044	0.074	0.016†
TLR6-S249P (%)				
Wild type	33/44 (75.0)	93/124 (75.0)	46 (75.4)	47/63 (74.6)
Heterozygous	11/44 (25.0)	30/124 (24.2)	14 (23.0)	16/63 (25.4)
Homozygous	0	1/124 (0.8)	1 (1.6)	0
Variant allele frequency	0.125	0.129	0.131	0.127
MAL-S180L (%)				
Wild type	33 (73.3)	103 (82.4)	53 (86.9)	50 (78.1)
Heterozygous	10 (22.2)	22 (17.6)	8 (13.1)	14 (21.9)
Homozygous	2 (4.4)	0*	0	0
Variant allele frequency	0.156	0.088	0.066	0.109

* Difference to healthy individuals ($P < 0.05$).
† Difference to asymptomatic infection ($P < 0.05$).

Here, we report frequencies from northern Chile of common innate immunity polymorphisms. In our study, a frequent MBL-deficiency allele shows borderline association with increased odds of CD, and the TLR4-deficiency allele D299G/T399I seems to protect against CCC. The small sample size of our study is an obvious limitation, particularly regarding the few healthy individuals who we were able to recruit (because of limited resources and reluctance to participate). We, thus, cannot exclude the possibility of type I statistical errors, and correction for multiple testing would render the already borderline associations insignificant. However, plausibility of hypotheses is also important for interpreting observed associations.[19] Multivariate analysis included age (and residence) but no other unknown confounders. We, therefore, consider our findings preliminary rather than definite, but they point to genetic aspects of innate immunity in CD that warrant replication in larger groups.

The complement system provides highly efficient protection against invading pathogens. MBL binds to the infectious metacyclic *T. cruzi* trypomastigotes, initiates (through activation of MBL-associated serine protease 2 [MASP-2]) the lectin pathway of early complement killing, and reduces cell invasion.[3] Low MBL levels should, thus, facilitate parasite survival and host cell invasion. In fact, CD patients in the present study were more likely to show the low MBL producer *MBL2*B* genotype than non-infected individuals. This finding accords with a recent study showing genotypes of low MASP-2 production to be overrepresented in CD patients.[20] However, *MBL2*B* did not influence manifestation, which contrasts findings from Brazil. In one study, MBL serum concentrations reportedly increased with increasing cardiac insufficiency,[7] suggesting ongoing inflammation. In a related study, low MASP-2 producer genotypes increased the risk of CCC.[20] These discrepant observations were attributed to differential roles of MBL and MASP-2 in phagocytosis, inflammation, and tissue damage.[20] In our study, neither MBL levels nor MASP-2 alleles were assessed, but it should be noted that MBL concentrations and actual activity do not necessarily correspond.[21] Also, differences in study design and activity of infection may be involved in the opposing findings from Brazil and Chile.

Increasing evidence supports a pathogenetic role of unbalanced proinflammatory responses in CCC.[1,4,8] Pre-disposition for curbed proinflammatory responses could, consequently, be beneficial, and in fact, *MAL/TIRAP* S108L has been associated with a reduced risk of CCC in Brazil.[8] MAL/TIRAP plays an essential role in signaling mediated by TLRs 1, 2, 4, and 6, which ultimately leads to proinflammatory cytokine release. In line with this role, heterozygous *MAL/TIRAP* S108L has been reported to confer protection against malaria, tuberculosis, pneumococcal disease, and bacteraemia.[22] We observed a marginal trend to reduced *MAL/TIRAP* S108L frequencies in CD patients but no differences between asymptomatic and CCC patients. Upstream, however, the common *TLR4*-deficiency allele D299G/T399I seemed to be involved in manifestation. TLR4 contributes to the recognition of *T. cruzi*: in deficient mice, parasite-specific chemokine production and neutrophil chemotaxis are reduced.[4] In humans, D299G/T399I reduces TLR4 responsiveness to lipopolysaccharide and associates with increased susceptibility and/or severity of numerous infectious and inflammatory diseases.[6] Heterozygosity for this main *TLR4*-deficiency allele showed a weak association with reduced odds of CCC, suggesting that a curtailed activation of this TLR pathway reduces tissue damage. In previous studies, this was not observed,[8] but our findings accord with the reported lack of effect on infection *per se*.[23] Our results also support the finding that common *TLR1* and *TLR2* SNPs do not seem to influence CD or CCC[8] and show the respective lack of impact for a frequent *TLR6* variant. The reason for the lack of impact remains unclear but may involve redundancy of TLR signaling.

In conclusion, disposition for low MBL levels seems to increase the odds of CD, which supports the role of the complement system in protecting against *T. cruzi* infection. TLR4 deficiency might be beneficial in preventing CCC.

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