Association of the Functional MICA-129 Polymorphism With the Severity of Chronic Chagas Heart Disease

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MICA-129 polymorphism affects the binding affinity of MICA molecules with the NKG2D receptor and influences effector cell function. The genotype met/met was associated with the severity of left ventricular systolic dysfunction (LVSD) in patients with chronic Chagas heart disease, while the val/val genotype was associated with the absence of LVSD.

Keywords. MICA polymorphism; Chagas disease; chronic Chagas heart disease; left ventricular systolic dysfunction.

Chronic Chagas heart disease (CCHD) is one of the severe clinical manifestations of Chagas disease. The condition manifests as heart failure, heart rhythm and electrical conduction disorders, thromboembolic events, precordial chest pain, and sudden death [1]. Patients with CCHD usually have a mononuclear cell infiltrate and interstitial and confluent myocardial fibrosis throughout the myocardium, accompanied by microvascular lesions [2], thus provoking left ventricular remodeling. However, the precise pathogenic mechanism of Chagas heart disease is not completely elucidated [3].

Received 18 April 2015; accepted 25 June 2015; electronically published 30 June 2015.

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Clinical Infectious Diseases® 2015;61(8):1310–3

DOI: 10.1093/cid/civ540

The MICA (major histocompatibility complex class I–related chain A gene) molecules are recognized by Tγδ and Tαβ lymphocytes, $CD8^+$ cells, and natural killer (NK) cells by the NKG2D receptors on their surfaces [4]. But, changing a single amino acid, a methionine (met) for a valine (val) at position 454 of exon 3, which corresponds to the amino acid 129 of the protein, changes MICA alleles from strong (MICA-129 met) to weak (MICA-129 val) binders of the NKG2D receptor; this very likely affects the activation of NK cells and the modulation of T cells [5].

The relationship between the MICA-129 polymorphism (rs1051792) and severity of CCHD remains unknown. Accordingly, we investigated the relationship between the MICA-129 met > val polymorphism (A > G) in exon 3 of the *MICA* gene and the severity of left ventricular systolic dysfunction (LVSD) in patients with CCHD.

METHODS

The Research Ethics Committee of the Medicine School in Sao Jose do Rio Preto (FAMERP - # 009/2011) approved this study. An informed consent form was signed by all participants.

A total of 189 consecutive unrelated male and female patients with CCHD, treated in the Cardiomyopathy Clinic of Hospital de Base of Fundação Faculdade de Medicina de São José do Rio Preto participated in this study. All patients underwent 2-dimensional echocardiogram. The severity of LVSD was graded according to left ventricular ejection fraction (LVEF) values measured with the Teichholz method according to the Brazilian guidelines of severe chronic heart disease [6]. Patients were classified into the following 3 groups according to LVEF: LVEF > 60% (patients without LVSD), LVEF between 60% and 40% (patients with mild to moderate LVSD), and LVEF < 40% (patients with severe LVSD) (Supplementary Table 1).

The laboratory diagnosis of Chagas disease was made by enzyme-linked immunosorbent assay (ELISA) of serum or plasma using the ELISAcruzi immunoassay (bioMerieux SA Brazil) and following the manufacturer's instructions.

Genomic DNA was extracted from peripheral blood using a silica-membrane column (PureLink, Genomic DNA Mini Kit, Invitrogen, Carlsbad, California) following the manufacturer's instructions. Verification of the MICA-129 polymorphism (A > G, rs1051792) in exon 3 was performed using nested polymerase chain reaction. The val-129 MICA allele was identified by the presence of a restriction site for the RsaI enzyme (FastDigest,

Table 1. MICA-129 Polymorphism in Patients With Chronic Chagas Heart Disease From Southeastern Brazil Using Recessive, Dominant, Additive, and Codominant Inheritance Models

MICA-129 Polymorphism	Severe LVSD n = 48 n (%)	Mild/Moderate LVSD n = 48 n (%)	Without LVSD (Normal) n = 93 n (%)	χ^2	*P* Value
Recessive inheritance model					
met/met	12 (25.0)	5 (10.4)	7 (7.5)	7.19	.007
met/val + val/val	36 (75.0)	43 (89.6)	86 (92.5)		
Dominant inheritance model					
met/met + met/val	36 (75.0)	28 (58.3)	48 (51.6)	9.02	.01
val/val	12 (25.0)	20 (41.7)	45 (48.4)		
Additive inheritance model					
met/met	12 (25.0)	5 (10.4)	7 (7.5)	12.36	.002
val/val	12 (25.0)	20 (41.7)	45 (48.4)		
Codominant inheritance model					
met/val	24 (50.0)	23 (48.0)	41 (44.1)	0.49	.78
met/met + val/val	24 (50.0)	25 (52.0)	52 (44.1)		
Genotype comparisons					
Recessive inheritance model					
Severe LVSD vs without LVSD (normal)				8.29	.004
Severe LVSD vs mild/moderate LVSD				3.50	.06
Mild/moderate LVSD vs without LVSD (normal)				0.33	.56
Dominant inheritance model					
Severe LVSD vs without LVSD (normal)				7.19	.007
Severe LVSD vs mild/moderate LVSD				3.00	.08
Mild/moderate LVSD vs without LVSD (normal)				0.57	.44
Additive inheritance model					
Severe LVSD vs without LVSD (normal)				9.82	.001
Severe LVSD vs mild/moderate LVSD				3.63	.06
Mild/moderate LVSD vs without LVSD (normal)				0.16	.68
Codominant inheritance model					
Severe LVSD vs without LVSD (normal)				0.23	.62
Severe LVSD vs mild/moderate LVSD				0.04	.83
Mild/moderate LVSD vs without LVSD (normal)				0.06	.79

Abbreviation: LVSD, left ventricular systolic dysfunction.

Thermo Scientific) created by a mismatch deliberately introduced into the nonsense primer [7] (Supplementary Table 2).

Groups were compared using the χ^2 test with Yates correction or Fisher exact test using the statistics program GraphPad Instat version 6.3 (http://www.graphpad.com/scientific-software/instat/). The genotype frequencies were also evaluated using the dominant (met/met + met/val vs val/val), recessive (met/met vs met/val + val/val), additive (met/met vs val/val), and codominant (met/val vs met/met + val/val) inheritance models. The odds ratio (OR) and the 95% confidence interval (CI) were calculated to determine the risk of developing LVSD. The Hardy–Weinberg equilibrium was verified using the ARLEQUIN program version 3.11 (http://cmpg.unibe.ch/software/arlequin3/). Differences in *P* values ≤.05 were considered statistically significant.

RESULTS

In this study population, the distributions of alleles associated with the MICA-129 polymorphism were in Hardy–Weinberg equilibrium ($P > .05$).

The data on genotype and allele frequencies are shown in Supplementary Table 3. The genotype met/met ($P = .007$; OR = 4.09; 95% CI, 1.49–11.24) and met allele ($P = .001$; OR = 2.38; 95% CI, 1.43–3.96) ($P = .04$; OR = 1.90; 95% CI, 1.06–3.41) were significantly associated with increased risk of

developing severe LVSD, while the MICA-129 val/val genotype ($P = .01$; OR = 0.35; 95% CI, .16–.76) and val allele ($P = .001$; OR = 0.41; 95% CI, .25–.69) ($P = .04$; OR = 0.52; 95% CI, .29–.93) were associated with a lower risk of developing severe LVSD.

The recessive and dominant inheritance models showed significant association between patients with severe LVSD and patients without LVSD ($P = .007$; $\chi^2 = 7.19$ and $P = .004$; $\chi^2 = 8.20$, respectively). The additive model also produced a significant association ($P = .001$; $\chi^2 = 9.82$), while the codominant model was nonsignificant ($P = .78$; $\chi^2 = 0.49$) as expected. Thus, the met > val polymorphism contributes to CCHD susceptibility under an additive model; individuals who are homozygous for the met allele have the highest risk of developing severe LVSD; those homozygous for the val allele seem to modify CCHD severity (Table 1).

DISCUSSION

To our knowledge, we are the first to address the importance of the functional MICA-129 polymorphism in the severity of LVEF in CCHD. LVEF is the powerful independent predictor of mortality for patients with chronic Chagas disease, in general [8], and one of the most important predictors of all-cause mortality in Chagas disease patients with chronic systolic heart failure in particular [9]. Therefore, the identification of *MICA* as a candidate gene of susceptibility in patients with these conditions has important implications for a better understanding of the immunopathogenesis of this disease.

Differences in the sequences of MICA alleles may influence the interaction of the MICA molecules with the NKG2D receptor. A study of the polymorphism at codon 129 showed that the alleles encoding methionine in this position have a considerably greater capacity to bind to the NKG2D receptor than alleles coding for valine in the same position [5]. Moreover, the interaction between NKG2D and the MICA molecules may potentially increase antitumor immune responses and participate in inflammatory processes with increases in the production of cytokines by NK cells. This interaction may also promote costimulatory signaling to specific $CD8^+$ T-cell self-antigens in autoimmunity [4].

Autoimmunity is accepted as one of the main pathogenic mechanisms responsible for CCHD [10]. Therefore, strong affinity formed by the NKG2D-MICA-129 met/met complex may be related to the tissue damage observed in CCHD due to a high activation of NK cells and $CD8^+$ T cells. The greater damage caused to the cardiac tissue can therefore develop into severe organ dysfunction. On the other hand, the homozygous presence of valine amino acid may generate a less pronounced immune response in cells, so that patients with this polymorphism are protected against the development of severe CCHD due to less affinity to the NKG2D immunoreceptor with consequent less tissue injury. Similar to the results found in this study, the MICA-129 polymorphism has been reported as a risk factor for the development of other autoimmune diseases [7, 11, 12].

Inflammatory infiltration in the chronic phase of Chagas disease presents with signs of cellular activity, with the main cells found being $CD4^+$ and $CD8^+$ T cells. However, NK cells, macrophages, and B lymphocytes are also present [13]. The function of NK cells and $CD8^+$ T cells in the elimination of transformed or stressed infected cells occurs together with self-tolerance, a property that is essential to prevent autoimmunity. Inappropriate expression of ligands for NK cell receptors leads to the activation of autoreactive effector cells and therefore can cause or exacerbate the autoimmunity [4]. It has been demonstrated that baseline levels of NK, NKT, and $CD4^+$ $CD25^{HIGH}$ cells with failed immunoregulation mechanisms associated with an increased expression of activated $CD8^+$ T cells are associated with cardiac events [14, 15].

The data obtained in this study indicated a possible susceptibility related to the homozygous genotype met/met with the severity of LVSD in the setting of CCHD, whereas the homozygous val/val seem to modify CCHD severity. Several studies have shown that uncontrolled activation of autoreactive effector cells may lead to tissue damage that, in turn, leads to the development of the clinical manifestations of severe CCHD [14, 15]. However, to better understand the role of cells expressing the NKG2D receptor as well to clarify the expression of MICA molecules in the immunopathogenesis of Chagas disease, others studies such as histopathological analysis in CCHD and cytotoxicity assays should be done.

CONCLUSION

Our data suggest that the homozygous met/met genotype may be related to a risk factor in the severity of LVSD observed in the setting of CCHD, while the homozygous val/val genotype was associated with protection against this condition in this study population.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors are grateful to all of the volunteers who participated in this study. Thanks to David Hewitt for his help with the English version.

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and do not necessarily reflect the views of the São Paulo Research Foundation (FAPESP).

Financial support. C. M. A. was supported by a grant from FAPESP (2013/06580-9); A. P. d. O. and A. V. d. S. C. were supported by a grant from the Brazilian Ministry of Education—Coordination of Improvement of Higher Education Personnel; L. C. d. M. was supported by grants from FAPESP (2013/25650-8, 2011/08075-4) and Faculdade de Medicina de São José do Rio Preto.

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Bestetti RB, Restini CBA. Precordial chest pain in patients with chronic Chagas disease. Int J Cardiol **2014**; 176:309–14.
2. Bestetti RB, Rossi MA. A rationale approach for mortality risk stratification in Chagas' heart disease. Int J Cardiol **1997**; 58:199–209.
3. Biolo A, Ribeiro AL, Clausell N. Chagas cardiomyopathy—where do we stand after a hundred years? Prog Cardiovasc Dis **2010**; 52:300–16.
4. Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science **1999**; 285:727–9.
5. Steinle A, LI P, Morris DL, et al. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics **2001**; 53:279–87.
6. Dutra OP, Besser HW, Tridapalli H, et al. Sociedade Brasileira de Cardiologia, II Brazilian guideline for severe heart disease. Arq Bras Cardiol **2006**; 87:223–32.
7. Amroun H, Djoudi H, Busson M, et al. Early-onset ankylosing spondylitis is associated with a functional MICA polymorphism. Hum Immunol **2005**; 66:1057–61.
8. Rassi A Jr, Rassi A, Rassi SG. Predictors of mortality in chronic Chagas disease: a systematic review of observational studies. Circulation **2007**; 115:1101–8.
9. Theodoropoulos TAD, Bestetti RB, Otaviano AP, Cordeiro JA, Rodrigues VC, Silva AC. Predictors of all-cause mortality in chronic Chagas' heart disease in the current era of heart failure therapy. Int J Cardiol **2008**; 128:22–9.
10. Cooke A, Zaccone P, Raine T, Phillips JM, Dunne DW. Infection and autoimmunity: are we winning the war, only to lose the peace? Trends Parasitol **2004**; 20:316–21.
11. Yoshida K, Komai K, Shiozawa K, et al. Role of the MICA polymorphism in systemic lupus erythematosus. Arthritis and Rheumatism **2011**; 63:3058–66.
12. Pollock RA, Chandran V, Pellett FJ, et al. The functional MICA-129 polymorphism is associated with skin but not joint manifestations of psoriatic disease independently of HLA-B and HLA-C. Tissue Antigens **2013**; 82:43–7.
13. Higuchi MD, Ries MM, Aiello VD, et al. Association of an increase in CD8+ T cells with the presence of *Trypanosoma cruzi* antigens in chronic, human, chagasic myocarditis. Am J Trop Med Hyg **1997**; 5:485–9.
14. Vitelli-Avelar DM, Sathler-Avelar R, Massara RL, et al. Are increased frequency of macrophage-like and natural killer (NK) cells, together with high levels of NKT and $CD4^{+}CD25^{high}$ T cells balancing activated $CD8^{+}$ T cells, the key to control Chagas' disease morbidity? Clin Exp Immunol **2006**; 145:81–92.
15. Sathler-Avelar R, Vitelli-Avelar DM, Teixeira-Carvalho A, Martins-Filho OA. Innate immunity and regulatory T-cells in human Chagas disease: what must be understood? Mem Inst Oswaldo Cruz **2009**; 104:246–51.