IL18 Gene Polymorphism Influences Age of Onset of DM1 in African Ancestry Brazilians

Alejandro Boëchat-Fernandes¹ Rosângela Roginski Réa² Nicole Balster Romanzini²
Marília Brito Gomes³ Lupe Furtado-Alle¹ Ricardo L. R. Souza¹

¹Department of Genetics, Federal University of Paraná, Curitiba, Paraná, Brazil

²Endocrinology and Metabolism Service, University Hospital of the Federal University of Paraná, Curitiba, Paraná, Brazil

³Diabetes Unit, Department of Internal Medicine, State University of Rio de Janeiro, Rio de Janeiro, Brazil

Address for correspondence Ricardo L. R. Souza, PhD, Department of Genetics, Federal University of Paraná, PO Box 19071, 81531-980 Curitiba, Paraná, Brazil (e-mail: ricardo.lehtonen@gmail.com).

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Abstract

Keywords

- type 1 diabetes mellitus
- *IL18*
- *IL12B*
- polymorphism analysis

The aim of this study was to investigate the relationship of two single nucleotide polymorphisms (SNPs) in the interleukin-18 (*IL18*) gene (rs187238, g.-137G > C; rs1946518, g.-607C > A) and one SNP of the *IL12B* gene (rs3212227 g.*159A > C, 3'UTR) with the age of onset for type 1 diabetes mellitus (DM1). A total of 1,101 patients with DM1 enrolled in 13 centers from different regions of Brazil were genotyped with TaqMan assay and classified according to the ancestry. Our results show that an SNP in *IL18* gene could be associated with DM1 age onset, taking into account that this studied variation affects gene expression.

Introduction

In present study, we investigate associations between interleukin-18 (*IL18*) (rs187238, g.-137G > C, and rs1946518, g.-607C > A) and *IL12B* (rs3212227, g.*159^a > C) genes and age of onset for type 1 diabetes mellitus (DM1) in a sample of 1,101 DM1 patients collected throughout 13 diabetes centers scattered in five regions of Brazil. The samples were collected by members of the Brazilian Type 1 Diabetes Study Group (BrazDiab1SG), listed in ►Appendix A.¹ Only patients with a clear diagnosis of DM1 at disease onset were included, with plasma glucose levels exceeding 200 mg/dL on more than one occasion, overt symptoms and signs of the disease and immediate insulin requirement and weight loss, polyuria, polydipsia, polyphagia. Included patients were at least 13 years of age, and were followed at each diabetes center for at least 6 months.

The DNA was extracted as previously described² and genotyped by TaqMan single nucleotide polymorphism (SNP) genotyping assays (Applied Biosystem). Individuals were classified as predominantly European ancestry (EUR),

African ancestry (AFR), or Native Amerindian (NAM), based in data from a previous study.² The classification of the three groups was done by selecting individuals above the 3rd quartile, so that there was no overlap. All the statistical analyzes were done with R program.³ Nonparametric data were compared by Mann–Whitney U test. Allele and genotype frequencies were compared by chi-square test. Genetic model was chosen through univariate regression and genetic influence on DM1 onset was verified by multivariate regression. Statistical power for regression analyses was 0.82. Median age of onset of DM1 was, respectively, 13.5, 14.0, and 12.5 years, and were significantly different between AFR and NAM ($p = 0.03$). In the three groups, 45% are males.

►Table 1 shows allele and genotype frequencies. For rs187238, the allele frequency was different only between EUR versus AFR ($p = 0.012$). The rs1946518 shows differences in allelic and genotypic frequencies between EUR versus AFR and AFR versus NAM ($p < 0.009$). The rs3212227 also shows differences in allele and genotype frequencies between EUR versus AFR and AFR versus NAM ($p < 0.039$), so the following analyses were done for each group separately.

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Table 1 Allele and genotype frequencies from SNPs rs187238, rs1946518, and rs3212227 in the three groups with different ancestry

		EUR (n = 267) A	AFR (n = 276) B	NAM (n = 268) C	A × B	p-Values ^a A × C	B × C
	G	0.66	0.73	0.68	0.012	0.559	0.054
<i>IL18</i>	C	0.34	0.27	0.32			
rs187238	GG	0.47	0.57	0.49	0.063	0.835	0.200
	GC	0.39	0.33	0.37			
	CC	0.14	0.10	0.14			
	C	0.52	0.62	0.52	0.002	0.996	0.002
<i>IL18</i>	A	0.48	0.38	0.48			
rs1946518	CC	0.27	0.38	0.29	0.009	0.817	0.008
	CA	0.49	0.47	0.46			
	AA	0.23	0.15	0.25			
	A	0.75	0.68	0.68	0.014	0.019	0.939
<i>IL12B</i>	C	0.25	0.32	0.32			
rs3212227	AA	0.58	0.42	0.46	0.001	0.039	0.191
	AC	0.35	0.52	0.45			
	CC	0.07	0.06	0.09			

Abbreviations: EUR: predominantly European, AFR: predominantly African, NAM: predominantly Native Amerindian; SNPs, single nucleotide polymorphisms.
^aAllele and genotype comparisons between groups using chi-square test.

Logistic regression analysis, using diagnostic age, divided by median, as dependent variable and genotypes as independent variables, showed a significant result ($p = 0.04$) for SNP rs1946518 from *IL18* gene in the AFR group. The A allele has a dominant effect on the predisposition to the early onset of DM1. The haplotypic analysis from *IL18* gene SNPs did not show significant results. This SNP is an eQTL and data at GTEx portal show the genotype effect on gene expression in pancreas. IL18 together with IL12 are proinflammatory cytokines which have been implicated in the pathogenesis of DM1.^{4,5}

IL18 is a cytokine that enhances both Th1 and Th2 immune responses.⁶ As an inflammatory cytokine, IL18 participates on the pathogenesis of DM1 together with IL12, inciting the response of Th1 lymphocytes. Because of a cascade effect, the production of IFN- γ modulates NK cells activities, increases tumor necrosis factor alpha and another cytokines (IL1) by the macrophages, adhesion molecules, and nitric oxide production are elevated in areas of chronic inflammation.^{7,8} Rise in IL18 level precedes development of the acute phase of autoimmune diseases similar to DM1.⁹

Another study showed that diabetes was diagnosed significantly earlier (at 16 years of age) in patients with at least three minor alleles (*IL18* g.-137C/ g.-607A /*IL12B* g.*159C), compared with those without these alleles (at 25 years of age). It was proposed that the age of diagnostics difference may depend on the synergic contribution of both genes.¹⁰ The intensity of β cell destruction, which influences the age of onset of DM1, is intertwined by genetic factors.¹¹ There are many studies on the genetic causes of DM1, but relatively few studies on the genetic influence on the age of onset of DM1 and the main results show the influence of major histocompatibility complex

(MHC) genes,^{12,13} so it is important to expand this search to better understand this aspect of the disease. The Brazilian population is mainly constituted by European, African, and Native American ancestry.² Due to this population stratification, we only detected association of the *IL18* gene in the group with predominantly African ancestry. A recent study,¹⁴ using genetic risk score, found differences on the age of diagnosis between Caucasians and Afro Americans, reinforcing the importance of ethnic origin, especially for admixed populations, such as the Brazilian population.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Authors' Contributions

Alejandro Boëchat-Fernandes: experimental data and analysis.
 Rosângela Roginski Réa: recruitment and diagnosis
 Nicole Balster Romanzini: recruitment and diagnosis
 Marília Brito Gomes: study design.
 Lupe Furtado-Alle: study design and analysis.
 Ricardo L. R. Souza: study supervision.

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Conflict of Interest

None declared.

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Appendix A Brazilian Type 1 Diabetes Study Group (BrazDiab1SG)

Executive steering committee: Marília Brito Gomes (chair), Carlos Antonio Negrato.

Principal investigators are indicated by an asterisk.

Department of Internal Medicine, Diabetes Unit, State University of Rio de Janeiro, Brazil: Marília Brito Gomes* (mariliabgomes@gmail.com).

Federal University Hospital of Rio de Janeiro: Melanie Rodacki*, M.D. (mrodacki2001@yahoo.com.br); Lenita Zaidenver, M.D. (lenitazaj@gmail.com) Joana Rodrigues Dantas, M.D. (joanardantasp@ig.com.br).

Diabetes Unit, University Hospital of São Paulo, São Paulo: Maria Lúcia Cardillo Corrêa-Giannella*, M.D. (malugia@lim25.fm.usp.br); Sharon Nina Admoni, M.D. (sharonadmoni@gmail.com); Daniele Pereira dos Santos, M.D. (dps.daniele@hotmail.com).

Bauru's Diabetics Association, Bauru, São Paulo: Carlos Antonio Negrato*, M.D. (carlosnegrato@uol.com.br); Maria de Fatima Guedes, M.D. (tatiguedes@hotmail.com).

Diabetes Unit, Federal University of São Paulo State, São Paulo: Sergio Atala Dib*, M.D. (sergio.dib@unifesp.br); Celso Ferreira de Camargo Sallum Filho, M.D. (celsosallum@superperig.com.br).

Diabetes Unit, University of Campinas, São Paulo: Elisabeth João Pavin*, M.D., (ejpavin@fcm.unicamp.br); Caroline Takano, M.D. (caroline.takano@gmail.com).

Clinical Hospital of the Federal University of Paraná: Rosângela Roginski Rea*, M.D. (rosangelarea@uol.com.br); Nicole Balster Romanzini, M.D. (nikbr@hotmail.com).

Clinical Hospital of Porto Alegre, Rio Grande do Sul: Mirela Azevedo*, M.D. (mirelajobimazevedo@gmail.com); Luis Henrique Canani, M.D. (luishenriquecanani@gmail.com).

Regional Hospital of Taguatinga, Brasília: Hermelinda Cordeiro Pedrosa*, M.D. (pedrosa.hc@globo.com); Monica Tolentino (monicatolentino@uol.com.br); Cejana Hamu Aguiar, M.D.

Diabetes and Endocrinology Center of Bahia: Reine Marie Chaves Fonseca*, M.D. (reinemar@terra.com.br); Ludmila Chaves Fonseca M.D., Raffaele Kasproicz, M.D. (raffaelebarros@hotmail.com).

Diabetes and Hypertension Center of Ceará: Adriana Costa e Forti*, M.D. (adrianaforti@uol.com.br); Angela Delmira Nunes Mendes, M.D. (angeladelmira@terra.com.br).

Federal University of Ceará: Renan Montenegro Junior*, M.D. (renanjr@ufc.br); Virgínia Oliveira Fernandes, M.D. (virginiafernande@hotmail.com).

Federal University Hospital of Pará: João Soares Felício*, M.D. (felicio.bel@terra.com.br); Flavia Marques Santos, M.D. (drafms@bol.com.br).