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ORIGINAL ARTICLE

Genetic variants in *DNMT1* and the risk of cardiac autonomic neuropathy in women with type 1 diabetes

Daniele Pereira Santos-Bezerra[1], Sharon Nina Admoni[1,2], Rosana Cristina Mori[3], Tatiana Souza Pelaes[1], Ricardo Vesoni Perez[1], Cleide Guimarães Machado[4], Maria Beatriz Monteiro[1], Maria Candida Parisi[5], Elizabeth Joao Pavin[5], Marcia Silva Queiroz[2], Marisa Passarelli[6], Ubiratan Fabres Machado[3], Maria Lucia Correa-Giannella[1,7]*

[1]Laboratory of Carbohydrates and Radioimuneassays (LIM-18), [2]Division of Endocrinology, Clinical Hospital, Medical School, [3]Department of Physiology and Biophysics, Institute of Biomedical Sciences, [4]Division of Oftalmology, Clinical Hospital, Medical School, University of Sao Paulo, Sao Paulo, [5]Department of Internal Medicine, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, [6]Laboratory of Carbohydrates and Radioimuneassays (LIM-18), Clinical Hospital, Medical School, University of Sao Paulo, and [7]Department of Post-graduation in Medicine, Nove de Julho University (UNINOVE), Sao Paulo, Brazil

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***Correspondence**
Maria Lucia Correa-Giannella
Tel.: +55-11-3061-8782
Fax: +55-11-3061-8453
E-mail address:
maria.giannella@fm.usp.br

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ABSTRACT

Aims/Introduction: Epigenetics participate in the pathogenesis of metabolic memory, a situation in which hyperglycemia exerts prolonged deleterious effects even after its normalization. We tested the hypothesis that genetic variants in an epigenetic gene could predispose to diabetes complications.

Material and Methods: We assessed the frequency of five single-nucleotide polymorphisms in the gene encoding deoxyribonucleic acid methytransferase 1 (*DNMT1;* rs8112895, rs7254567, rs11085721, rs17291414 and rs10854076), and their associations with diabetic kidney disease, retinopathy, distal polyneuropathy and autonomic cardiovascular neuropathy in 359 individuals with long-term type 1 diabetes.

Results: None of the single-nucleotide polymorphisms studied was significantly associated with the presence of chronic complications in the overall population. However, after sex stratification, the minor allele C of rs11085721 conferred risk for cardiovascular neuropathy in women after adjustment for confounding variables (odds ratio 2.32; 95% confidence interval 1.26–4.33; $P = 0.006$).

Conclusions: The fact that heterozygous mutations in *DNMT1* are associated with hereditary sensory autonomic neuropathy provides plausibility to the present finding. If confirmed in independent samples, it suggests that genetic variants in epigenetic genes might predispose to more or fewer epigenetic changes in the face of similar metabolic derangements triggered by hyperglycemia, constituting the "genetics of epigenetics" for microvascular diabetes complications.

INTRODUCTION

Epigenetic changes participate in the pathogenesis of diabetes complications; periods of hyperglycemia cause permanent abnormalities, such as aberrant gene expression, in target tissues of complications. This phenomenon is called metabolic memory and explains why hyperglycemia exerts persistent deleterious effects even after its normalization[1].

Deoxyribonucleic acid methyltransferases (DNMTs) add a methyl group at cytosine residues located in CpG islands from the promoter regions of genes, resulting in gene silencing[2]. Our literature search did not reveal studies evaluating the association between diabetes complications and *DNMT* polymorphisms.

To investigate the participation of "genetics of epigenetics" in diabetes complications, we evaluated the association between *DNMT1* single-nucleotide polymorphisms (SNPs) and diabetic kidney disease (DKD), retinopathy (DR), distal polyneuropathy and cardiovascular autonomic neuropathy (CAN). The minor allele of one of the evaluated SNPs (rs11085721) conferred risk for CAN in women, suggesting that genetic variants in epigenetic genes participate in the susceptibility to this complication.

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METHODS

Participants

A total of 359 individuals with type 1 diabetes were recruited from two university hospitals. Type 1 diabetes was diagnosed in the presence of hyperglycemia, undetectable C-peptide or ketoacidosis and positive autoantibodies (glutamic acid decarboxylase, islet cell antibodies or tyrosine phosphatase-like insulinoma antigen 2). All participants were under permanent insulin treatment initiated at diagnosis or within 6 months of diagnosis. Inclusion criteria were type 1 diabetes duration ≥10 years and glycated hemoglobin >8% in any period of life. Exclusion criteria were hypocortisolism and use of medications interfering with heart rate. The study was carried out in compliance with the Declaration of Helsinki, in accordance with institutional ethics committees.

After signing informed consent, participants were evaluated for clinical and biochemical characteristics, and for the status of DKD, DR, polyneuropathy and CAN, as previously described[3]. Briefly, DKD was diagnosed in the presence of macroalbuminuria (albumin : creatinine ratio >300 mg/g) and/or reduced estimated glomerular filtration rate (<60 mL/min 1.73/m^2, as calculated by Chronic Kidney Disease Epidemiology Collaboration[4]). Standardized seven-field retinal color photographs with the use of TRC-NW8 Non-Mydriatic Retinal Camera (Topcon, Oakland, NJ, USA) was used for the diagnosis of DR; the images were evaluated by a single ophthalmologist trained in DR following the international classification of DR[5]. Polyneuropathy was diagnosed by the sum of the Neuropathy Symptoms Score and Modified Neuropathy Disability Score[6]. CAN was diagnosed based on Ewing tests, spectral analysis of the heart rate variability and systolic blood pressure after 3 min standing. Seven variables were evaluated: (i) spectral power in the low frequency band; (ii) spectral power in the high-frequency band; (iii) spectral power in the very low-frequency band; (iv) maximum/minimum 30:15 ratio; (v) expiration : inspiration ratio; (vi) Valsalva ratio; and (vii) postural change in systolic blood pressure. Diagnosis of CAN was made in the presence of three or more abnormal tests[7].

Genotype Analysis

Deoxyribonucleic acid extraction from peripheral blood leukocytes was carried out by a salting-out procedure[8]. To cover most of the genetic variability across the extended region of

Table 1 | Characteristics of individuals with type 1 diabetes (overall and sorted according to the presence or absence of cardiovascular autonomic neuropathy)

	Overall	Without CAN	With CAN	*P*-value
Clinical and biochemical characteristics				
n	359	238	121	
Age (years)	36 (29–45)	37 (29–46)	35 (30–44)	0.39
Sex, female (%)	59.6	59.2	60.3	0.35
BMI (kg/m^2)	24.1 (21.7–26.8)	24 (22–27)	24 (21–28)	0.78
Arterial hypertension (%)	50.1	42	67	<0.0001
Dyslipidemia (%)	46	43	52	0.19
Total cholesterol (mg/dL)	173 (148–195)	171 (149–192)	177 (145–215)	0.17
HDL cholesterol (mg/dL)	58 (46–71)	59 (47–73)	58 (46–67)	0.37
LDL cholesterol (mg/dL)	94 (80–110)	94 (80–107)	95 (77–124)	0.45
Triglycerides (mg/dL)	78 (58–107)	76 (57–98)	90 (63–130)	0.0004
eGFR (mL/min.1.73 m^2)	97 (64–112)	100 (78–115)	81 (16–106)	<0.0001
Diabetes status				
Diabetes duration (years)	23 (18–30)	23 (18–30)	24 (18–30)	0.99
Age at diagnosis (years)	12 (7–18)	12 (7–19)	11 (6–18)	0.25
HbA1c (%)	8.2 (7.4–9.4)	8.1 (7.3–9.0)	8.5 (7.5–10)	0.004
Fructosamine (μmol/L)	369 (322–430)	367 (322–415)	384 (319–481)	0.15
Microvascular complications				
Diabetic kidney disease (%)	29.8	20	52.3	<0.0001
Retinopathy (any degree) (%)	69	64.7	79.3	0.01
Distal polyneuropathy (%)	34.5	26.7	57	<0.0001
Use of medicines				
ACEI (%)	35	33	42	0.01
Statin (%)	45	41	52	0.02

Results expressed as median and interquartile range, and percentage of cases (categorical variables). Glycated hemoglobin (HbA1c) is expressed as the percentage of total hemoglobin. $P \leq 0.05$ was considered significant. ACEI, angiotensin-converting enzyme inhibitors; BMI, body mass index; eGFR, estimated glomerular filtration rate computed with the Chronic Kidney Disease Epidemiology Collaboration equation; CAN, cardiac autonomic neuropathy; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

DNMT1 (including regions spanning 15 kb upstream and downstream of the gene), five tag SNPs were chosen based on the Haploview program (http://www.broadinstitute.org/scientificcommunity/science/programs/medical-and-populationgenetics/haploview/haploview) and on HapMap (http://hapmap.ncbi.nlm.nih.gov), using a pair-wise approach, a $r^2 \geq 0.9$ and a minor allele frequency of >0.05: rs8112895, rs7254567, rs11085721, rs17291414 and rs10854076. Based on Haploreg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), rs8112895 and rs10854076 are intronic SNPs, whereas the remaining are non-intronic SNPs: rs7254567 and rs11085721 are located 12 kb 5′ of *DNMT1*, and rs17291414 is located 11 kb 5′ of *DNMT1*.

SNPs were genotyped by real-time polymerase chain reaction (StepOne Plus; Applied Biosystems, Foster City, CA, USA), using predesigned Human TaqMan Genotyping Assays 40X: C__29287779_10 (rs8112895), C__34042774_10 (rs17291414), C__31764973_10 (rs11085721), C__26941571_10 (rs7254567) and C___1142308_10 (rs10854076; Thermo Fisher Scientific, Waltham, MA, USA). The genotyping success rate was ~99% for all SNPs, and SNP/sample call rates were >99%.The Hardy–Weinberg equilibrium was tested for all SNPs, and the only one with a *P*-value <0.05 was rs8112895, which was not considered in the association analysis.

Statistical Analysis

Differences between groups were assessed by Pearson's χ^2-test and Wilcoxon/Mann–Whitney tests (nominal and continuous variables, respectively). The associations of the genotypes (co-dominant model) with complications were assessed by logistic regression analyses to compute odds ratios and 95% confidence intervals. Adjustments for confounding variables were carried out by including them as covariates in the regressive model. Interaction between genotype and sex was evaluated by including a "crossed" compound covariate (sex/genotype) in the regression models. The stratification by sex was then carried out by nesting the genotype variable within the sex variable in the analysis model. This results in the computation of statistical effects for men and women separately. Correction for multiple comparisons due to multiple SNP testing took into account the effective number of independent tests (Meff) based on the degree of linkage disequilibrium between SNPs, and $P < 0.01$ was considered significant[9]. The power to detect associations with CAN was 84% for rs11085721 in the female population. Statistics were carried out with the JMP software (SAS Institute, Cary, NC, USA).

RESULTS

The characteristics of the individuals with type 1 diabetes (overall and sorted according to status of CAN, the only complication associated with one of the evaluated SNPs) are shown in Table 1. A total of 121 out of 359 participants (33.7%) had the clinical form of CAN (≥3 altered tests). Individuals with CAN, when compared with individuals without CAN, presented a higher prevalence of hypertension, higher triglyceride and glycated hemoglobin concentrations, and lower estimated glomerular filtration rate. DKD, DR, polyneuropathy, and use of angiotensin-converting enzyme inhibitors and of statins were more frequent in the group presenting CAN.

Table 2 | Genotype frequencies of four single-nucleotide polymorphisms in *DNMT1* according to the status of cardiovascular autonomic neuropathy in the overall individuals with type 1 diabetes

SNP	Without CAN	With CAN	OR (95% CI)	*P*-value
n	238	121		
DNMT1				
rs17291414				
GG	0.570	0.534	1.07 (0.61–1.43)	0.73
GA	0.343	0.375		
AA	0.087	0.091		
MAF	0.258	0.278		
rs11085721				
GG	0.713	0.591	1.50 (0.59–1.72)	0.08
GC	0.266	0.375		
CC	0.021	0.033		
MAF	0.154	0.221		
rs7254567				
GG	0.362	0.273	1.19 (0.55–1.45)	0.38
GA	0.473	0.568		
AA	0.165	0.159		
MAF	0.401	0.443		
rs10854076				
GG	0.787	0.750	1.26 (0.49–1.65)	0.70
GC	0.193	0.239		
CC	0.020	0.011		
MAF	0.116	0.130		

Odds ratio (OR) for the minor allele determined in logistic regression in a co-dominant model analysis adjusted for glycated hemoglobin, triglyceride concentrations, estimated glomerular filtration rate, the presence of hypertension and use of medicines (angiotensin-converting enzyme inhibitors and statins). $P \leq 0.01$ is considered significant. CAN, cardiovascular autonomic neuropathy; CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

None of the studied SNPs was significantly associated with the presence of the evaluated chronic complications in the overall population (data shown for CAN in Table 2, data for DKD, DR and distal polyneuropathy are available as Supporting Information in Tables S1, S2 and S3, respectively). SNP rs11085721 showed a significant interaction with sex ($P = 0.005$). After sex stratification, the minor allele C of this SNP conferred risk for CAN in women with type 1 diabetes after adjustment for glycated hemoglobin, triglycerides, estimated glomerular filtration rate, the presence of hypertension and use of medicines (angiotensin-converting enzyme inhibitors and statin; odds ratio 2.32, 95% confidence interval 1.26–4.33; $P = 0.006$; Table 3).

Table 3 | Genotypes frequencies of rs11085721 in *DNMT1* according to the status of cardiovascular autonomic neuropathy in individuals with type 1 diabetes sorted by sex.

rs11085721	Without CAN	With CAN	OR (95% CI)	*P*-value
DNMT1				
Female T1D				
n	141	72	2.32 (1.26–4.33)	0.006
GG	0.702	0.514		
GC	0.277	0.458		
CC	0.021	0.028		
MAF	0.169	0.250		
Male T1D				
n	97	49	0.89 (0.28–2.59)	0.84
GG	0.773	0.794		
GC	0.213	0.206		
CC	0.014	0.000		
MAF	0.120	0.103		

Odds ratio (OR) for the minor allele determined in logistic regression in a co-dominant model analyses adjusted for glycated hemoglobin, triglycerides, estimated glomerular filtration rate, the presence of hypertension and use of medicines (angiotensin-converting enzyme inhibitors and statins). $P \leq 0.01$ is considered significant. Power of analysis: 0.84. CAN, cardiovascular autonomic neuropathy; CI, confidence interval; MAF, minor allele frequency; T1D, type 1 diabetes.

DISCUSSION

This is one of the rare studies showing the association of a SNP in a gene involved in epigenetic modification with diabetes complication. The presence of the minor allele C of rs11085721 in *DNMT1* conferred risk for CAN in women with type 1 diabetes.

CAN is one of the least recognized diabetes complications, even though it can have serious consequences; it affects the autonomic nerves that innervate heart and blood vessels, causing sympathovagal imbalance, heart rate deregulation and cardiac impairment. CAN is also an independent predictor factor for cardiovascular mortality and progression of DKD[10].

As for other diabetes complications, poor glycemic control is the most important risk factor for CAN development and progression. After 14 years of follow up of the Diabetes Control and Complications Trial cohort in the Epidemiology of Diabetes Interventions and Complications study, a higher incidence of CAN was observed in the group exposed to suboptimal metabolic control (conventional therapy during the Diabetes Control and Complications Trial), showing the persistent deleterious effects of hyperglycemia. This finding supports the impact of metabolic memory on CAN development, which was not as evident for distal polyneuropathy[11,12].

The fact that heterozygous mutations in *DNMT1* are associated with hereditary sensory autonomic neuropathy[13] provides plausibility to our finding. However, it is interesting that rs11085721 conferred susceptibility for CAN, but not for peripheral neuropathy. This SNP has previously been associated with increased susceptibility to acute lymphoblastic leukemia[14] and to an increased risk of attempted suicide only in women[15], but there are no functional studies reported for it. Thus, it is not possible to know whether rs11085721 confers susceptibility to CAN or if it is in linkage disequilibrium with the susceptibility SNP.

We do not have an explanation for the sex-specific association, but sexual dimorphism is reported in several pathological conditions and might result from sex hormone-induced differences in the epigenetic status of key genes[16]. It is worth mentioning that animal studies have shown greater DNMT1 activity in males than in females[17].

Despite the ethnic admixture that characterizes the Brazilian population, the minor allele frequency observed in this series (0.1765) is similar to that reported in the National Center for Biotechnology Information Single Nucleotide Polymorphism Database for Caucasian populations (0.144–0.166), which decreases the probability of the observed result being a false positive association. However, the present finding must be interpreted in the context of limitations of cross-sectional studies. If confirmed in larger and independent samples, it suggests that genetic variants in epigenetic genes might predispose to more or fewer epigenetic changes in the face of similar metabolic derangements triggered by hyperglycemia, constituting the "genetics of epigenetics" for microvascular diabetes complications.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Genotype frequencies of four single-nucleotide polymorphisms in *DNMT1* according to the status of diabetic kidney disease in the overall individuals with type 1 diabetes.

Table S2 | Genotype frequencies of four single-nucleotide polymorphisms in *DNMT1* according to the status of diabetic retinopathy in the overall individuals with type 1 diabetes.

Table S3 | Genotype frequencies of four single-nucleotide polymorphisms in *DNMT1* according to the status of diabetic distal polyneuropathy in the overall individuals with type 1 diabetes.