Genetic variants in \( \text{DNMT1} \) 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\(^7\), Maria Lucia Correa-Giannella\(^{1,7,*}\)

\(^1\)Laboratory of Carbohydrates and Radioimmunoeassays (LIM-18), \(^2\)Division of Endocrinology, Clinical Hospital, Medical School, \(^3\)Department of Physiology and Biophysics, Institute of Biomedical Sciences, \(^4\)Division of Ophthalmology, 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 Radioimmunoeassays (LIM-18), Clinical Hospital, Medical School, University of Sao Paulo, and \(^7\)Department of Post-graduation in Medicine, Novo de Julho University (UNINOVE), Sao Paulo, Brazil

Keywords
Chronic complications, Epigenetics, Metabolic memory

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

J Diabetes Investig 2019; 10: 985–989
doi: 10.1111/jdi.12988

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 methyltransferase 1 (\( \text{DNMT1} \); 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 \( \text{DNMT1} \) 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 (\( \text{DNMTs} \)) 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 \( \text{DNMT} \) polymorphisms.

To investigate the participation of “genetics of epigenetics” in diabetes complications, we evaluated the association between \( \text{DNMT1} \) 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.
RESULTS

There were 359 eligible patients who were recruited from two university hospitals. Of these, 121 had type 2 diabetes and 238 had type 1 diabetes (Table 1). There were no significant differences in age, sex, obesity, hypertension, and dyslipidemia between the two groups. Overall, type 1 diabetes patients had a significantly higher estimated glomerular filtration rate than the type 2 diabetes patients (60.3 vs. 59.2 mL/min/1.73 m², respectively, P = 0.05).

Several complications were more frequent in the type 1 diabetes patients. Antihypertensive and lipid lowering medication use was higher in the type 1 diabetes patients (P = 0.02 and 0.05, respectively) (Table 1). Furthermore, type 1 diabetes patients had a higher prevalence of distal polyneuropathy (57% vs. 26.7%, P < 0.0001), retinopathy (79.3% vs. 64.7%, P = 0.01), and diabetic kidney disease (52.3% vs. 20%, P < 0.0001) than the type 2 diabetes patients.

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

| Characteristic                          | Overall | Without CAN | With CAN | P-value |
|----------------------------------------|---------|-------------|----------|---------|
| 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/m²)                            | 24.1 (21.7–26.8) | 24 (22–27) | 24 (21–28) | 0.78 |
| Arterial hypertension (%)              | 50.1 | 43 | 52 | 0.19 |
| 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 m²)                  | 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 ≤ 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/science/community/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 SNP/sample call rates were >99%. The Hardy–Weinberg equilibrium was tested for all SNPs, and SNP/sample call rates were >99%. The Hardy–Weinberg equilibrium was tested for all SNPs, and SNP/sample call rates were >99%. The Hardy–Weinberg equilibrium was tested for all SNPs, and SNP/sample call rates were >99%

### Statistical Analysis

Differences between groups were assessed by Pearson’s $\chi^2$-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 regression 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 statins were more frequent in the group presenting CAN.

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 statins). $P \leq 0.01$ is considered significant. CAN, cardiovascular autonomic neuropathy; CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

### 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 |
|-----------|-------------|----------|-------------|---------|
| rs17291414| n            | n         |             |         |
| 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| n            | n         |             |         |
| 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 | n            | n         |             |         |
| 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| n            | n         |             |         |
| 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.
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 |
|------------|-------------|----------|-------------|---------|
|            | 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 ≤ 0.01 is considered significant. Power of analysis: 0.84.

**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 DKD10.

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 polyneuropathy11,12.

The fact that heterozygous mutations in DNMT1 are associated with hereditary sensory autonomic neuropathy13 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 leukemia14 and to an increased risk of attempted suicide only in women15, 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 genes16. It is worth mentioning that animal studies have shown greater DNMT1 activity in males than in females17.

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.

**ACKNOWLEDGMENT**

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) under Grant # 2012/04831-1. Marisa Passarelli, Ubiratan Fabres Machado, and Maria Lucia Correa-Giannella are recipients of a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**DISCLOSURE**

The authors declare no conflict of interest.

**REFERENCES**

1. Prattichizzo F, Giuliani A, Ceka A, et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics 2015; 7: 56.
2. Shen L, Kondo Y, Guo Y, et al. Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. PLoS Genet 2007; 3: 2023–2036.
3. Monteiro MB, Santos-Bezerra DP, Thieme K, et al. Thioredoxin interacting protein expression in the urinary sediment associates with renal function decline in type 1 diabetes. Free Radic Res 2016; 50: 101–110.
4. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604–612.
5. Wilkinson CP, Ferris FL 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003; 110: 1677–1682.
6. Meijer JW, Bosma E, Lefrandt JD, et al. Clinical diagnosis of diabetic polyneuropathy with the diabetic neuropathy symptom and diabetic neuropathy examination scores. *Diabetes Care* 2003; 26: 697–701.

7. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; 115: 387–397.

8. Lahiri DK, Nurnberger JL. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; 19: 5444.

9. Gelius-Dietrich G, Desouki AA, Fritzemeier CJ, et al. Sybil: efficient constraint-based modelling in R. *BMC Syst Biol* 2013; 7: 125.

10. Jaiswal M, Divers J, Urbina EM, et al. Cardiovascular autonomic neuropathy in adolescents and young adults with type 1 and type 2 diabetes: The SEARCH for Diabetes in Youth Cohort Study. *Pediatr Diabetes* 2018; 19: 680–689.

11. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia* 1998; 41: 416–423.

12. Pop-Busui R, Herman WH, Feldman EL, et al. DCCT and EDIC studies in type 1 diabetes: lessons for diabetic neuropathy regarding metabolic memory and natural history. *Curr Diab Rep* 2010; 10: 276–282.

13. Baets J, Duan X, Wu Y, et al. Defects of mutant DNMT1 are linked to a spectrum of neurological disorders. *Brain* 2015; 138: 845–861.

14. Luo Y, Yu L, Yu T, et al. The association of DNA methyltransferase 1 gene polymorphisms with susceptibility to childhood acute lymphoblastic leukemia. *Biomed Pharmacother* 2015; 73: 35–39.

15. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted suicide. *Mol Psychiatry* 2012; 17: 433–444.

16. Ptak C, Petronis A. Epigenetic approaches to psychiatric disorders. *Dialogues Clin Neurosci* 2010; 12: 25–35.

17. McCarthy MM, Nugent BM. The epigenetics of sex differences in the brain. *J Neurosci* 2009; 29: 12815–12823.

**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.